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ANNUAL REPORT OF PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

JULY 1, 1975 THROUGH JUNE 30, 1976

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ANNUAL REPORT OF PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
JULY 1, 1975 THROUGH JUNE 30, 1976

The National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports a broad program of research directed at improved methods for the diagnosis, treatment and prevention of allergies and other disorders arising from deranged immunologic function and of infections.

The major events involving the Director's Office were the retirement of the former Director, Dr. Dorland J. Davis on August 1, 1975, the appointment of a new Director, Dr. Richard M. Krause on November 1, 1975 and the appointment of Dr. John R. Seal as Deputy Director, also on November 1. Dr. Krause was a Professor at Rockefeller University in New York City prior to his move to the NIH.

A second major event of the year which involved the Director's Office was the occurence of an outbreak of influenza at Fort Dix, New Jersey caused by a swine-like influenza A virus (HswN1). Beginning in February 1976 both the Director and Deputy Director were involved in the series of conferences and departmental meetings which led to congressional appropriation for the National Influenza Immunization Program. Subsequently the Deputy Director took responsibility for the Infectious Diseases Branch of the Collaborative Research Program in the implementation of research related to the special funding under the program. This research involved the testing of the vaccines to obtain needed data on reactions and antigenicity, surveillance for the occurrence in various population groups of influenza caused by HswN1 or other influenza viruses, and follow-up of vaccine recipients for late adverse effects or efficiency of vaccines. It also included the testing of experimental vaccine preparations.

Contractors already engaged in epidemiologic and clinical studies of respiratory diseases responded quickly to the need to test vaccines and between April 15, when the National Influenza Immunization Program (NIIP) became official and by June 21, more than 6,000 persons were given the vaccines from the four manufacturers. Of these about 700 were studied by the CDC, another 700 by the military, and the balance either at the NIH or by NIAID contractors.

On June 21, in a public meeting at the NIH and jointly sponsored by the NIAID, CDC and BoB/FDA, the results of these tests were presented by the participants in the trials. This was followed by a media interview reported on national television. The results made it clear that persons 25 years of age or older could be satisfactorily immunized with a dose of vaccine less than had been anticipated. Also that children, although responding well to the whole virus vaccine, had too many reactions to any of the doses tested for these dosages to be used in mass immunization of normal children (although satisfactory for high risk children if needed). Additional studies in children were immediately begun by NIAID contractors. The data from the initial trials were summarized in the Journal of Infectious Diseases for July 1976. The June issue carried a

summary of the events leading to the NIIP.

The Director participated in meetings of the Commissions on Arthritis and Diabetes. The Office prepared and submitted summary documents on NIAID research activities related to arthritis, diabetes, lupus erythematosis, cystic fibrosis and renal diseases. He also participated in meetings of the Cystic Fibrosis Foundation, American Thoracic Society, Lupus Erythematosis Association, and had other discussions with voluntary health associations and professional societies related to the NIAID. The Director also met many speaking commitments incurred prior to joining the NIH which occasioned travel to meetings in the United States and abroad.

Plans for reorganization of the Institute's extramural programs along programmatic lines were prepared and submitted. Recruitment of a new Scientific Director was vigorously pursued as was recruitment of new leadership for the extramural programs. Although considerable progress was made, none of these efforts had reached fruition by the end of the fiscal year.

The Institute's viral hepatitis research program was selected for OPS tracking by the NIH for the year. This led to quarterly meetings with the Director and Senior Staff of the NIH at which various aspects of the system were reported. These reports included discussions of the problems involved in initiating studies of a hepatitis B vaccine in volunteers which related to the concerns of the DHEW on recompense to subjects injured during the course of clinical research and provided a case in point of future research needs.

The Office of Research Reporting and Public Response (ORRPR) prepared 28 "Research Reports" during the year. These non-technical summaries are based on papers or presentations by NIAID scientists, grantees and contractors. They are distributed to the Institute's professional staff, members of the Institute's advisory groups and to science writers on request. They often are incorporated into more lengthy Institute reports, and are sometimes used to answer inquiries from the medical profession, science writers, and the general public.

In addition to the three special reports regularly submitted to the Congressional Appropriations Committees ("Veneral Diseases," "Acute Respiratory Diseases," and "Clinical Immunology: Allergy and Organ Transplantation"), the ORRPR for the first time prepared major segments for three additional Congressional reports -- "Arthritis," "Cystic Fibrosis," and "Kidney Disease."

The ORRPR has continued its publication efforts to "tell the story" of allergic, immunologic and infectious disease research. Two new publications were added to the allergy series -- "Mold Allergy" and "Poison Ivy Allergy." There are now six pamphlets in this series. In addition to sending copies of the series to members of the American Academy of Allergy, ORRPR introduced the series to 14,000 of the Nation's pediatricians with the suggestion that they or the parents of their patients may wish to obtain copies from the Superintendent of Documents, GPO.

"Miscellaneous Microbes - the Nonconformists," -- the third in a series of pamphlets to interest high school students in science and possible careers in medicine and the health sciences -- had its initial distribution this year. Its author, Elaine Wilson, received an award of excellence in "Writing for a non-professional audience" from the American Medical Writers Association.

The Institute's first pamphlet on "Sexually Transmitted Diseases" was produced as part of the Infectious Disease series, and the "flu" pamphlet was revised to include information about "swine-like" flu.

ORRPR is distributing 20 fact sheets on infectious disease research. Seven were updated this year and one new fact sheet, "Trypanosomiasis," was written.

The distribution of ORRPR publications has steadily increased from approximately 18,000 in FY 1972 to more than 160,000 this year. Most of these publications are sent in response to requests from the public. However, 18,000 additional copies of our "Mononucleosis" fact sheet were sent to the Consumer Products Information Center, GSA, to supplement last year's request for 25,000. There was a greater demand than GSA had anticipated.

In another light, the NIH received 5 awards for excellence in the Federal Design Council's Design Response competition. Three of the five NIH awards were for NIAID publications: "The U.S.-Japan Cooperative Medical Science Program," "Allergy Pamphlet Series," and "Infectious Diseases Pamphlet Series."

The Institute participated in the NIH Bicentennial Open House on May 1 and 2. Dr. Brian Murphy delivered a public lecture on influenza research and three exhibits were manned by Institute scientists and ORRPR staff. One exhibit highlighted the current clinical aspects of the Laboratory of Infectious Disease (research on hepatitis, influenza and diarrhea). A second exhibit of the Laboratory of Parasitic Disease focused mainly on a videotape recording of the malaria parasite invading a red blood cell. And the third exhibit illustrated research conducted at several of the NIAID-supported Allergic Disease Centers.

The ORRPR also developed an exhibit, "NIAID-Serving Professionals and the Public", which could be tailored to specific audiences. This year it was used by the Research Resources Branch and ORRPR to display reagents and catalogs as well as ORRPR publications at the annual meetings of the American Academy in San Juan and the American Society for Microbiology in Atlantic City. It also was used by the Transplantation Immunology Branch for a presentation on the Institute's Serum Bank for the annual meeting of the American Association for Clinical Histocompatability Testing.

As in the past 5 years, the ORRPR cooperated with the American Academy of Allergy to hold a briefing for science writers in conjunction with the Academy's annual meeting. This year scientists speaking from San Juan reported on several newsworthy topics -- new drugs for asthma, a new hay fever preparation, insect allergy, food allergy, and exercise-induced asthma -- to writers attending the San Juan meeting as well as via two-way telephone hook-up to NIAID's conference room in Bethesda.

The greatest demand of staff time with representatives of the news media had to do with the swine influenza program, particularly during April through June. The Information Officer represented NIH in planning sessions with the CDC, FDA and the Public Affairs Office of the Assistant Secretary for Health. A formal briefing was held to kick off the first vaccine trial at the NIH Clinical Center. Another briefing was tied to the June 21 meeting announcing results of the first trials.

Operation of the Freedom of Information Act has taken additional staff time, particularly in the extramural area of the Institute. Most of the requests have been for minutes of advisory meetings or documents relating to Institute research grants (applications, summary statements, progress reports, etc.). In only one case has the Institute and NIH requested a denial, actually a partial denial, of a Freedom of Information request.

Conference support continued to be managed by the Office of Special Programs and Projects. A total of 29 prospecti were received during the fiscal year. The total cost of these 29 conferences was described as $1.579 million with $672 thousand being requested in NIH support. After review in the NIAID and other appropriate Institutes, the NIAID approved funding for 20 of the 29 to a total of $289 thousand, about $54,000 of which was to be obligated in the transitional quarter. Other Institutes contributed $149 thousand. A listing of the approved conferences follows:

1. Role of Products of the Histocompatibility Gene Complex
2. VI International Symposium on Streptococcal Pyogenes
3. Workshop on Design & Testing of Safer Prokaryotic Vehicles & Bacterial Hosts for Research on Recombinant DNA Molecules
4. 20th Annual Conference of Institute of Polio & Virus Encephalitides
5. 6th International Congress of the Transplantation Society
6. Workshop on the Antigens in Hypersensitivity Pneumonitis
7. Conference on Animal Virology
8. Gordon Conference on Microbial Toxins & Pathogenicity
9. National Conference on Scabies & Pediculosis
10. Workshop on Nature & Mechanics of Action of Ir Genes
11. Vth International Congress of Protozoology
12. Current Status & Prospects for Improved and New Bacterial Vaccines
13. 1st Symposium on Biology of Non-gonococcal Urethritis
14. Workshop on Combined Immune Deficiency: Its Nature & Treatment
15. 3rd International Congress of Immunology
16. Workshop on Advanced Bacterial Genetics: Construction & Use of Recombinant DNA Molecules
17. 11th Leukocyte Culture Conference
18. 3rd International Colloquium on Rhabdoviruses
19. International Symposium on HLA and Disease

This office also continued to manage the NIAID PL480 agreements, foreign

quarantine permit issuance, and publication of Scientific Memoranda. Scientific Memoranda enabling rapid exchange of data and information between participating scientists on an international basis, have proven to be a useful mechanism. During the year a new one was begun, the Nucleic Acid Recombinant Scientific Memorandum. This was in response to the growing interest in this area and was one of the routes used in dissemination of Guidelines.

NIAID scientists and staff were recognized for excellence. Heading the list was the election of Dr. Wallace P. Rowe to the National Academy of Science and his receipt of the Selman A. Waksman Award. Dr. Robert M. Chanock received the Modern Medicine Magazine Award For Distinguished Achievement. Dr. Edgar Ribi of the Rocky Mountain Laboratory and Leon Rosen of the Laboratory of Parasitic Diseases received the DHEW Distinguished Service Award. Drs. Rose Mage and Ira Green received the PHS Superior Service Award. Drs. Malcolm Martin, Milton Puziss, Phillip J. Baker, Luz Froehlich and Janice Hartley received the NIH Director's Award. Dr. James Rose received the PHS Meritorious Service Medal. Drs. Robert Gerloff and Allen P. Kaplan received the PHS Commendation Medal. Dr. John R. Seal was honored with a unique affair sponsored by the Medical Alumni Association and Medical School of the University of Virginia. "John Seal Day" included a scientific program reviewing research with which Dr. Seal had been associated, cocktails in the garden of Dr. Kenneth Crispell, Vice President for Medical Affairs of the University, and an elegant dinner in the Rotunda.

There were many accomplishments during the year. These are reflected in the following pages reflecting activities in the program areas.

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REPORT OF THE ASSOCIATE DIRECTOR

Introduction

Fiscal Year 1976 was characterized by an extreme shortage of funds for competing research grants. This resulted in a number of disappointments in the immunology, viral and bacterial diseases programs, through the transfer of many important projects to other institutes which are more adequately funded. This deficiency in funds was brought about not only by limited appropriations but also by a high noncompeting research grants base created in Fiscal Years 1974 and 1975 when considerable new money became available at the end of the Fiscal Year. Even with the modest increase included in the FY 76 appropriation for research grants, less than 20 percent of all applications which competed for FY 76 research support could be funded. The fellowship and training programs fared a little better; however, new awards in these areas had to be held up until the end of the fourth quarter pending clarification of the funding authority and final allotment.

The Extramural Program began preparations for a reorganization to begin with Fiscal Year 1977. While this reorganization made numerous inconveniences and was disadvantageous to several staff members, they unanimously undertook the chores associated with reorganization with good spirit and have greeted each new change with resourcefulness and loyalty to the Institute's programs and goals. As Associate Director it has been a pleasure to be working with such a versatile and industrious staff. There seems to be no question at this time that the reorganization will be carried out smoothly and successfully.

Program evaluation activity which was reported so extensively in last year's Annual Report was not followed vigorously this year because of changes in program emphasis and because of the reorganization of the Institute. It is expected that in Fiscal Year 1977 this activity can continue under the new standing committees which will be managed by Extramural Program operations.

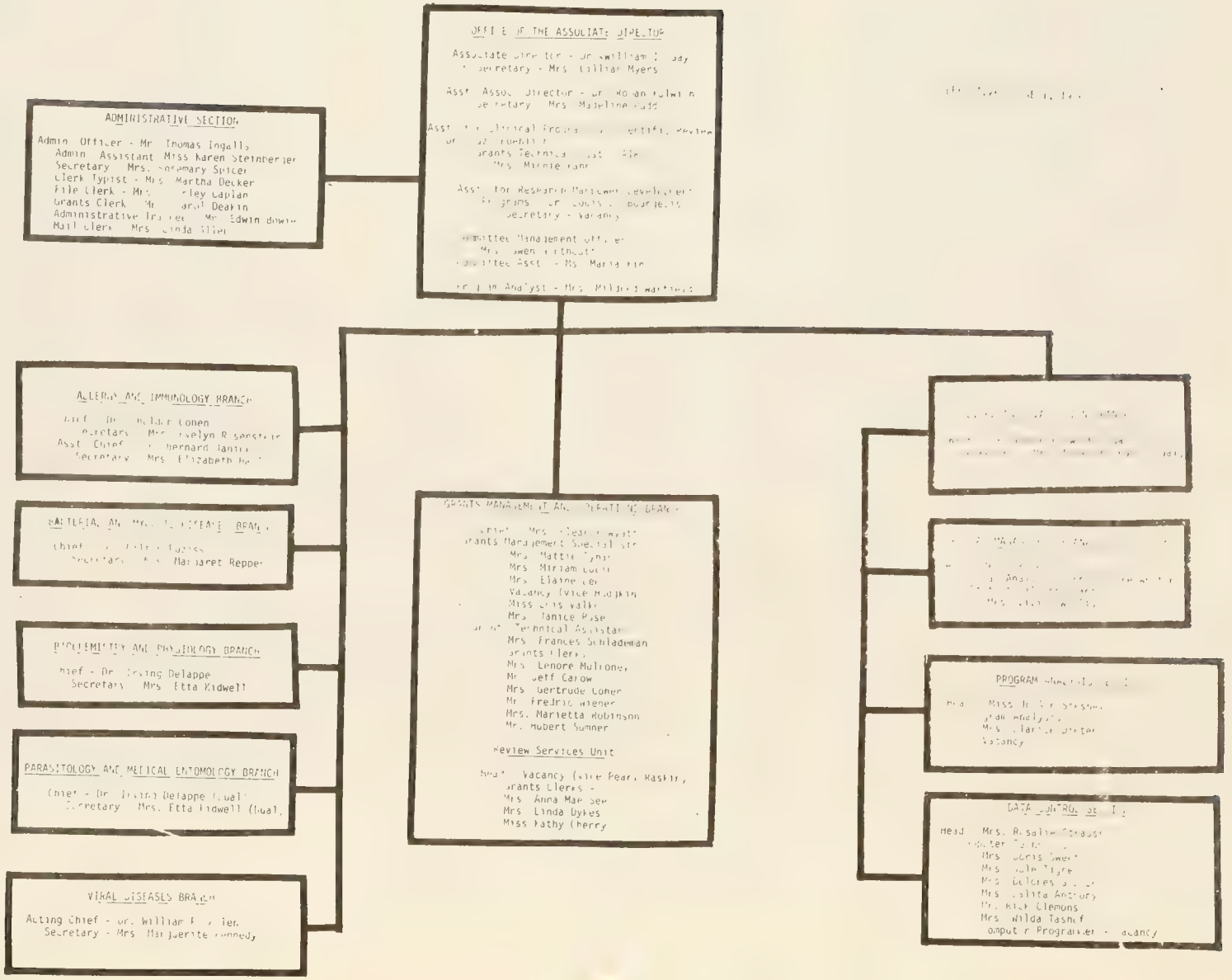
The present organization of extramural programs along program lines has been in existence for eleven years. It was organized in its present structure by Dr. James Colbert. The present and final staffing in this structure is shown in Figure 1. The planned organizational structure will include all extramural management functions, (program analysis, grants management, contract management), and extramural resources (training and the research resources branch).

Program Analysis

The Program Analysis Section, Review and Evaluation Branch, with the assistance of the Data Control Section, continues to supply an increasing amount of programmatic data and analyses, not only to the NIAID for the purpose of evaluation and management of current and projected operating

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

EXTRAMURAL PROGRAMS


OFFICE OF THE ASSOCIATE DIRECTOR
Associate Director - Dr. William E. Gaudy
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Asst. Assoc. Director - Dr. Roland Kolwyck
Secretary - Mrs. Madeline Judd
Asst. for Clinical Programs - Dr. Gerald Levine
Grants Technical Assistant
Mrs. Minnie Kane
Asst. for Research Management Development Programs - Dr. Louis A. Bourgeois
Secretary - Vacancy
Committee Management Officer
Mrs. Owen Kirkhart
Committee Asst. - Ms. Maria Kim
Program Analyst - Mrs. Mildred Martinez
ADMINISTRATIVE SECTION
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Admin. Assistant - Miss Karen Steinberger
Secretary - Mrs. Rosemary Spicer
Clerk Typist - Mrs. Martha Decker
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Administrative Trainee - Mr. Edwin Bowie
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Asst. Chief - Dr. Bernard Jaffee
Secretary - Mrs. Elizabeth Hall
BACTERIAL AND MYCOTIC DISEASES BRANCH
Chief - Dr. Peter Figures
Secretary - Mrs. Margaret Reppert
BIOCHEMISTRY AND PHYSIOLOGY BRANCH
Chief - Dr. Irving Delappe
Secretary - Mrs. Etta Kidwell
PARASITOLOGY AND MEDICAL ENTOMOLOGY BRANCH
Chief - Dr. Irving Delappe (Dual)
Secretary - Mrs. Etta Kidwell (Dual)
VIRAL DISEASES BRANCH
Acting Chief - Dr. William P. Glezen
Secretary - Mrs. Marguerite Kennedy
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Grants Management Specialists
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Mrs. Miriam Cohen
Mrs. Elaine Lee
Vacancy (Vice McGuinn)
Miss Doris Walker
Mrs. Janice Rose
Grants Technical Assistant
Mrs. Frances Schladweiler
Grants Clerks
Mrs. Lenore Mulrone
Mr. Jeff Carow
Mrs. Gertrude Cohen
Mr. Fredric Wiener
Mrs. Marietta Robinson
Mr. Hubert Sumner
Review Services Unit
Head - Vacancy (Vice Pearl Haskin)
Grants Clerks -
Mrs. Anna Mae Lee
Mrs. Linda Dykes
Miss Kathy Cherry
PROGRAM
Head - Miss Ruth Kirkpatrick
Program Analysts
Mrs. Clarice Porter
Vacancy
DATA CONTROL UNIT
Head - Mrs. Rosalie Strauss
Computer Technician
Mrs. Doris Sweet
Mrs. Julie Tighe
Mrs. Delores Baker
Mrs. Calista Anthony
Mr. Rick Clemons
Mrs. Wilda Tashof
Computer Programmer - Vacancy

Figure 1

programs, but also to other B/I/D's at the NIH, the DHEW, other federal and state agencies, and members of Congress. For example, during the past year, programmatic data has been provided to: Dr. M. L. Furcolow, consultant on mycology research and training for a review of fungal diseases as a national problem; to extramural program administrators for reviews of research supported by NIAID grants and contracts relevant to arthritis and other connective tissue diseases, diseases of the lung, kidney and urinary tract, cardiovascular system, and skin; and to the Rand Corporation for a study of NIH-supported research citations and research publications. Updating of the annual NIH Clinical Trial Inventory continues to represent a substantial investment of professional effort in the area of program analysis: at least two person months in FY 1975.

During the past year the Data Control Section, in addition to providing data for reporting programmatic, budgetary and management activities to all areas of the Extramural Programs, initiated a new system to capture and retrieve active and pending contract data and to generate monthly reports. In collaboration with the Allergy and Immunology Branch, a computerized system has been designed and implemented which is able to capture and retrieve specialized programmatic data relative to allergic, immunologic, and infectious diseases from competing NIAID awards.

Fiscal Management and Analysis

The Fiscal Management and Analysis Section, Review and Evaluation Branch, was established in July 1975 to analyze fiscal data, prepare financial reports, develop funding plans, assist with preparation and execution of the EP-NIAID budget, and perform accounting services for the extramural programs.

The EP budget for FY 76 presented some serious problems in funding regular research grants. The non-competing base had increased significantly due to the release for payment of a large number of competing applications late in FY 75. Since there was no provision made in the FY 76 President's Budget to cover this extra commitment, funds planned for support of competing grants had to be used to meet non-competing obligations. See Tables I and II.

Starting with FY 77, the government-wide fiscal year will begin October 1 instead of July 1. To accommodate this change-over, FY 76 has been extended from 12 to 15 months in length, with a separate appropriation to meet the obligations occurring in the "transition quarter."

TABLE I

EP-NIAID COMPETING GRANTS AND AWARDS
(Reviewed/Approved/Funded)

	FY 1975	FY 1976[1/] (Est'd)
RESEARCH GRANTS:		
Reviewed	1041	1031
Approved	856	834
Funded	399	157
Percent of Approvals Funded	46.6%	18.8%
CAREER/ACADEMIC AWARDS:		
Reviewed	100	69
Approved	69	48
Funded	28	5
FELLOWSHIPS (INDIVIDUAL):		
Reviewed	429	249
Approved	399	224
Funded	174	105
FELLOWSHIPS (INSTITUTIONAL):		
Reviewed	16	54
Approved	13	49
Funded	8	16

1/ Does not include applications reviewed for or paid during Transition Quarter (July 1-Sept 30, 1976)

TABLE II

PROGRAM ANALYSIS

FY 1976 - REGULAR RESEARCH GRANTS[1/]
(Dollars in thousands)

PROGRAMS	TOTAL AMOUNT[2/]
ALLERGIC AND IMMUNOLOGIC DISEASES:	$ 28,200
Asthma and Allergic Diseases	1,750
(Asthma and Allergic Disease Centers.....$ 1,280)	
Clinical Immunology and Immunopathology	5,640
Selective Immunologic Activation	2,890
Lymphocyte Biology	1,700
BACTERIAL AND FUNGAL DISEASES:	19,900
Biology of Sexually-Transmitted (Venereal) Diseases	2,900
Bacterial Diseases Preventable by Vaccines	3,230
Hospital-Associated Infections	1,660
Mechanisms of Resistance to Antimicrobial Agents	3,030
VIRAL DISEASES:	15,100
Influenza and other Respiratory Diseases	1,210
Viral Hepatitis	750
Antiviral Substances	1,450
Clinical Virology	650
Chronic and Degenerative Diseases of Man	925
PARASITIC DISEASES:	6,937
Immunology of Parasitic Infections	1,870
Biological Regulation of Vectors	1,990
TOTAL REGULAR RESEARCH GRANTS	$ 70,137

1/ Does not include amounts paid in the transition quarter (7/1-9/30/76)

2/ Estimated as of 4/23/76

Committee Management

The Federal Advisory Committee Act, the Freedom of Information Act, and the Privacy Act continue to impose regulations and restrictions within which all advisory committees are required to function. During the past year, all of these regulations have been adhered to in the use of groups which are advisory to the Institute.

The National Advisory Allergy and Infectious Diseases Council and the Allergy and Immunology Research Committee are now functioning with full complements of authorized members. Nominations have been made for vacancies being created on the Council by members whose terms end in 1976. All appointments have been made for vacancies being created on the Allergy and Immunology Research Committee during 1976.

Human Subjects

As was indicated in the last Annual Report many new policies and procedures have emerged in the last few years. Their chief intent is to assure that human subjects involved in NIH-supported biomedical research are adequately protected. These procedures are being gradually implemented, and NIH staff as well as peer review groups are closely monitoring their implementation. As a routine post-Council activity, NIAID-EP staff informs the Office for Protection From Research Risks, via the Assistant for Clinical Programs, of Council and staff actions on all applications identified by study sections as involving potential or actual risk to human subjects. Each summary statement now contains a phrase indicating whether or not human subjects are involved and if further action is required on the former. The problem of the timely submission to NIH of the Human Subject Certification has not been settled, but we expect policy to be set on this in the near future.

The National Commission for the Protection of Human Subjects is continuing public hearings on the use of special groups in research, including prisoners, fetuses, children, and institutionalized mentally infirm. The NIAID, along with other BIDs, has submitted a report to the Commission about the types of research it is currently supporting which involves children, as part of the material to be used at its public hearings.

Other human subject issues are emerging and are under study, including indemnification of injured research subjects, public announcement of proposed and about-to-be-funded clinical trials in order to elicit public input and comment, and a hard look into the use of prisoners as human volunteers.

The inventory of NIH-supported clinical trials has been refined and updated, and the updating will be a continuing, annual activity.

The NIAID has also actively participated in a Committee that has formulated recommendations for an effective coordination of clinical programs among BIDs, and between BIDs and the General Clinical Research Center Program of DRR.

DAPA Task Force

An NIAID Advisory Council Task Force was appointed as a result of Council action in June 1975 for the purpose of developing a statement of policy regarding the mechanism of the Dollar Allocation for Program Areas (DAPA) system which has been used by NIAID since June 1971 as a system for preferential funding. Discussion papers were presented at the March 1976 Council meeting on the questions of who should exercise control in selecting research funds for special emphasis, and should there be a mechanism for preferential funding based on high program relevance. An analysis of the effect of past preferential funding is underway, and the Advisory Council will consider in the future the question of what alternative procedures might be used for preferential funding, as well as the criteria to be used for evaluating the effects of preferential funding.

National Arthritis Commission

The National Arthritis Act was passed in January 1975 and funds were finally made available for operation of the Commission in June of 1975. The Director of NIAID is an ex officio member of this Commission; however, at the time the Commission was formed, Dr. Davis was close to retirement and Dr. Krause's appointment was not yet confirmed. Dr. Gay was asked to represent the Director of NIAID in this capacity.

The NIAID is involved in arthritis studies through its support of research in infectious diseases and in immunology. The National Arthritis Act recognized this and made the Director of NIAID an ex officio member. (The Director of the National Institute of General Medical Sciences is also an ex officio member.) The NIAID is the second largest supporter of research related to arthritis at the National Institutes of Health. It supports approximately $8 million in research grants, largely in immunology.

Biohazards

During this Fiscal Year, the problem of potential biohazards emanating from recombinant nucleic acid manipulations has been discussed in a series of meetings of the NIH Recombinant DNA Molecule Advisory Committee which should, in the next few weeks, produce guidelines concerning this type of experimentation.

NIAID Extramural Programs has been represented by Dr. Delappe who has attended all of these meetings.

In December, 1975 our Institute was the first to send out a modification of a letter prepared by Dr. Leon Jacobs which is serving as an interim measure until more specific guidelines regarding possible biohazards are formulated. We have received responses pledging cooperation from all of the recipients who are or may, in the future, be conducting experiments involving the cloning of biochemically produced recombinant nucleic acid molecules.

In addition, prior to payment, we have sent a number of research grants involving cloning to the NIH Recombinant DNA Molecule Advisory Committee for their opinion concerning the safety of the experiments involved despite the fact that these grants had received prior peer review.

It is proposed that grants involving cloning be separated from the existing Mechanisms of Resistance to Antimicrobial Agents program and form a new program with goals and content based on recombinant nucleic acid research.

While these events have taken place in Extramural Programs other progress related to cloning has been achieved under the aegis of Dr. John Nutter in the contracts area, including the La Jolla, California, workshop and the review of contract proposals for safer cloning vehicles (plasmids and bacteriophage) and bacterial hosts.

Training and Fellowships

To determine the degree of effort of the National Institute of Allergy and Infectious Diseases in the area of training and fellowship support, it would appear to be expedient to examine those programs being phased out, and to note by comparison the effect of the new National Research Service Award Act of 1974, which superceded them.

Under the authority of the PHS Act, Title III, Public Law 78-410 (1970) as amended, there were still active 73 "T01" programs last fiscal year. This year there were 50 such programs supported with about 225 trainees. Only 22 T01 programs will be supported in FY 1977. These will have a total of approximately 100 trainees.

The other fellowship programs which were authorized under P.L. 78-410 and designated "F02" and "F03" have been terminated. The "F02" program provided support for postdoctoral research training in health related areas. The "F03" special fellowships program supported individuals generally in mid-career to extend their potential for health and health-related areas.

The (F22)-Individual Post Doctoral Fellowships and the (T22) Institutional Post Doctoral Fellowships also known as the "Weinberger Programs" are also in the process of being phased out. This was the last year in which the NIAID supported the remaining eight (8)

institutional (T22) training grants. These training grants have a total of 22 trainees. Five of the eight programs were training in the area of sexually transmitted diseases (STD). There are 32 individual fellows being supported in the F22 program. Five of these will be carried over and funded in the transitional quarter. In FY 1977, only four fellows will be supported in this program.

There are eight post doctoral training grants being funded under the National Research Service Act of 1974--PL 93-348. Three of these grants are for training in STD and the balance are for training in allergy and immunology. There are 19 recommended trainees, but only 12 have been appointed in these eight programs. It is projected that about 16 training grants may be supported after the 1976 June Council. This will represent a potential pool of approximately 56 trainees. There are 166 active individual fellows under the NRSA. These were non-competing in FY 76. It is interesting to note that training in immunology are 64 fellows, in microbiology and mycology 56, in virology 32, and in parasitology 14.

It is projected that 70-80 more individual fellows will be funded later this fiscal year. Sixteen fellows are now in the process of receiving support.

During the fiscal year, there were a number of (F32) individual awards which were not accepted by the applicants. The reasons for non-acceptance were not given, therefore, these can be placed in a broad category of non-acceptance, early terminations or withdrawals.

The breakdown for non-acceptance by program area is as follows:

		Number Activated
Allergy	1	13
Immunology	14	43
Bacterial Physiology	0	13
Parasitology	1	12
Virology	8	36
Bacteriology & Mycology	11	33
Total	35	150

Eleven of those who activated terminated early.

One of the probable reasons for non-acceptance may be due in part to the differences between Congress and the Administration which has caused long delays in the passage of appropriations and legislation which has made planning difficult and engendered a feeling of

uncertainty in the successful competitors of the fellowships. Fellows, therefore, seek elsewhere for support and opportunities to pursue their careers.

Figure 2 depicts competing and non-competing postdoctoral fellowships from 1969 through 1975, and Figure 3 shows predoctoral trainees supported by the T01 institutional training grants. Please note that in the latter graph, the number shown reflects both part-time and full-time trainees. The graph shows the phaseout of the T01 programs in FY 1978. Those trainees remaining in the T01 program are added to the trainees that will be supported under the NRSA authority. The T01 grants are in phaseout and this is reflected in the projected years 1976-1978.

It is interesting to note that although the number of non-competing postdoctorals maintained approximately the same level from 1969 through 1973, the same period reflects a drop in competing applications supported. The competition reached a new low in 1973, the year of impounded funds. Fiscal Year 1974 shows a dramatic decrease in non-competing fellows which was the effect of the number of funded competing proposals from 1969-1973. In that same year (F.Y. 1974), the competing applications increased. This no doubt was due to the release of impounded funds. The effect of inflation coupled with budgetary constraints is shown both in the competing and non-competing areas. Again it should be noted that in F.Y. 1976, the number of competings supported is greater than the number of non-competings. This is due to the new program (the NRSA) while the old programs, particularly the T01, are being phased out.

Personnel

Late in the fiscal year several EP offices were physically relocated within the Westwood Building and most of the Institute's Collaborative Program personnel were moved from Building 31 to Westwood. These changes are the first step in effecting the planned reorganization of the Institute.

The Extramural Programs was authorized an increase of three full-time positions to the personnel ceiling. This increase was necessary because of the additional workload in recent years. (See Figure 4)

One of the new positions was used to cover a ceiling average as a result of the appointment of a statistical assistant in our Fiscal Management and Analysis Section. Another position was used to hire a committee management assistant to help with added responsibilities as a result of the centralization of the Institute's committee management functions. The third position was used to hire a secretary for Dr. Louis Bourgeois, Assistant for Manpower Development Programs, who has been sharing a secretary since October 1974. We also hired an additional part-time person to assist the Data Control Section in

Figure 2

Competing and Non-competing Postdoctorals
From 1969 - 1978*

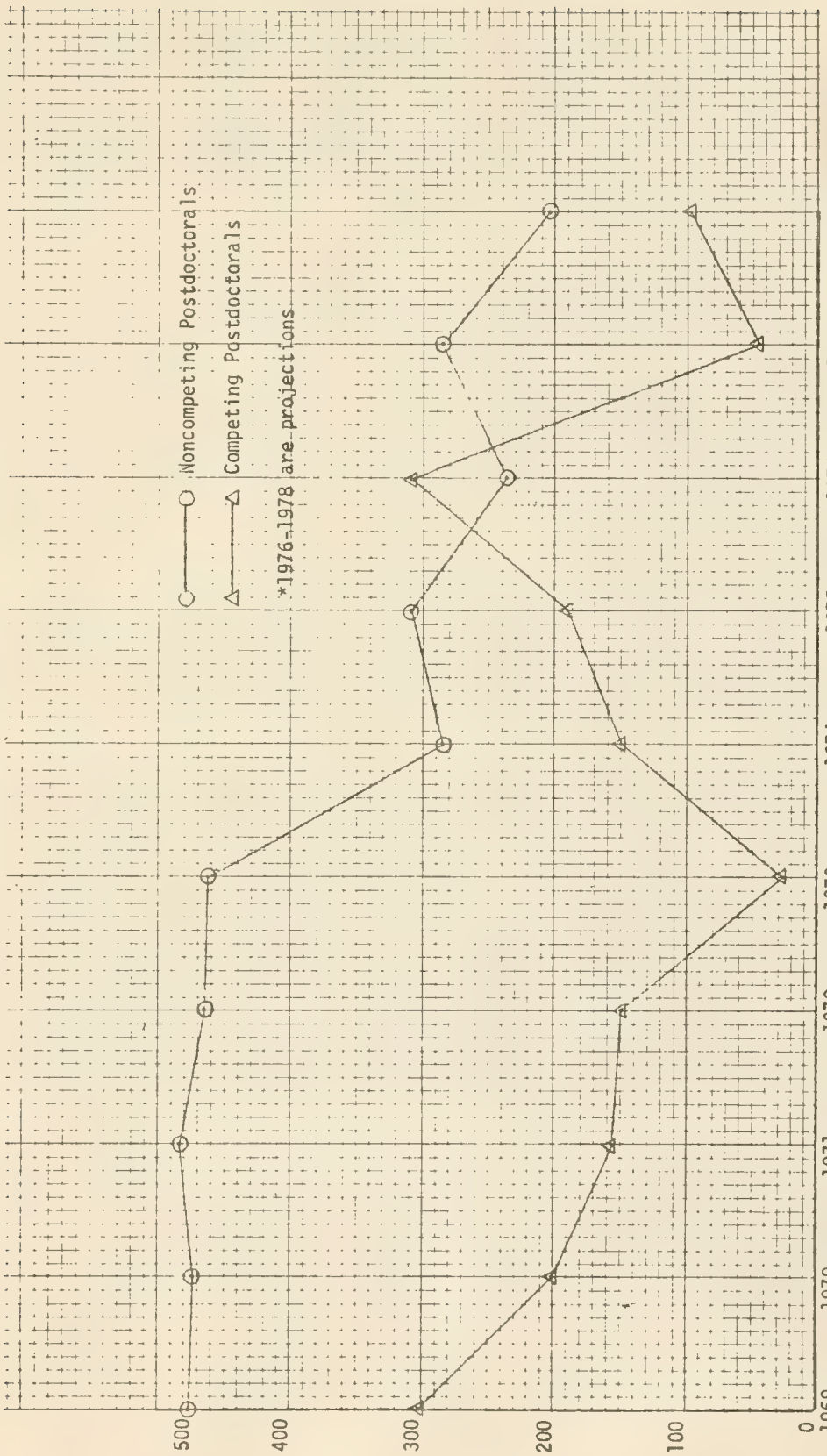
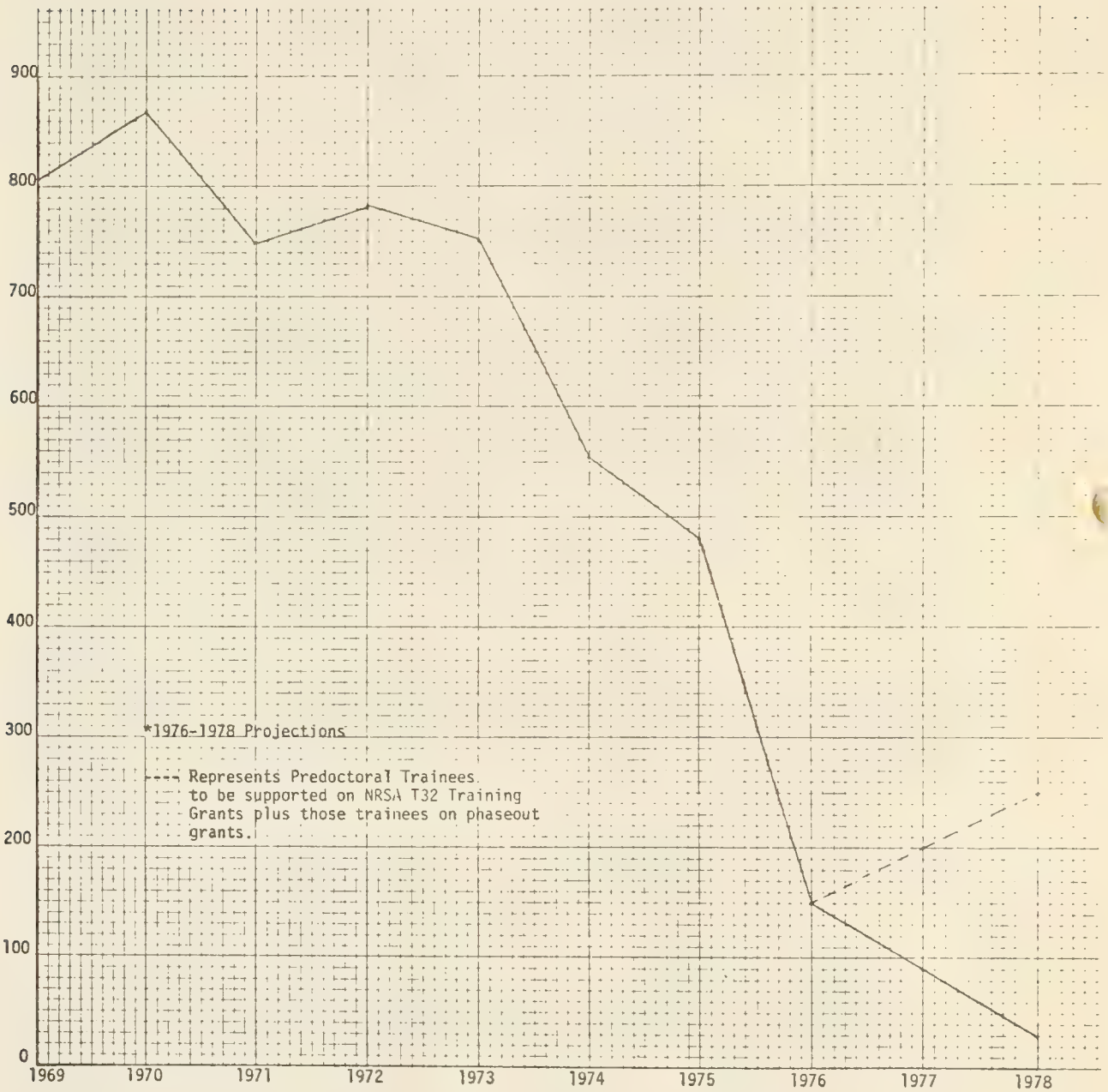


Figure 3

Predoctoral Trainees Supported on T01 Training Grants
From 1969 - 1978*



maintaining the Institute's contract data as part of a coordinated effort in reporting NIAID support of all extramural activities.

The Grants Management and Operations Branch has had notable turnover during the last year. Actions include the hiring of two new grants clerks and two grants management specialists. In addition, Pearl Raskin retired in May 1976. Mrs. Raskin had worked in the Extramural Programs since April 1961 and had served as head of the GMOB Review Unit for nearly ten years.

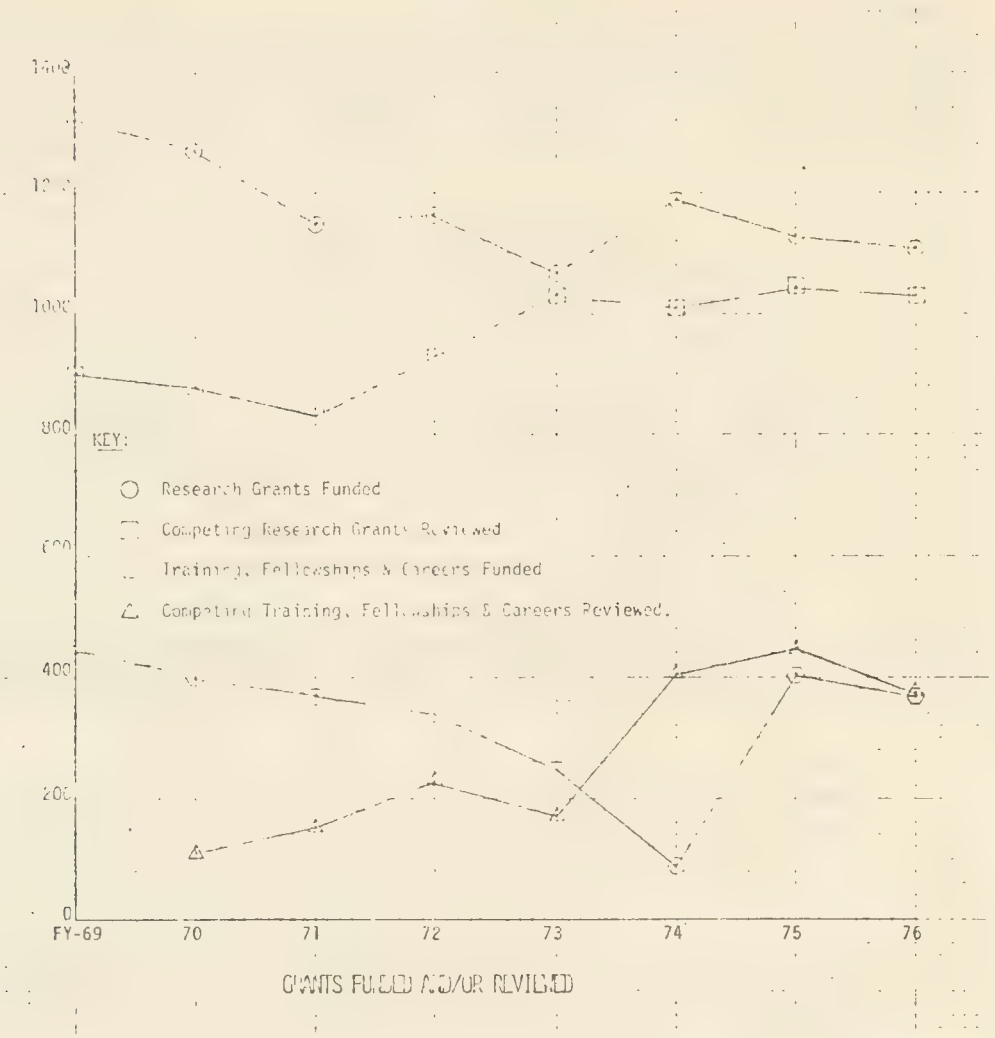
In December, Dr. Clarence Sooter retired from the PHS Commissioned Corps after six years as Chief of the Viral Diseases Branch. Dr. William Allen, who has been assisting in the Branch since February 1975, was appointed Acting Chief. Dr. Allen had previously engaged in viral research and served as Head of the Pathobiology Division and Virology Department at the Delta Regional Primate Research Center, Tulane University. He had also served as Chief, Virology IV Branch, Virus and Rickettsia Division, U.S. Army Biological Laboratories, Fort Detrick from 1967 to 1971.

Extramural Program employees attended forty-nine training courses at a cost of $3,546. The cost does not include the salaries while training nor support of the STEP courses or the Upward Mobility Program which are funded centrally. Doris Sweet and Delores Gibson received awards for outstanding academic achievement in the Upward Mobility Program.

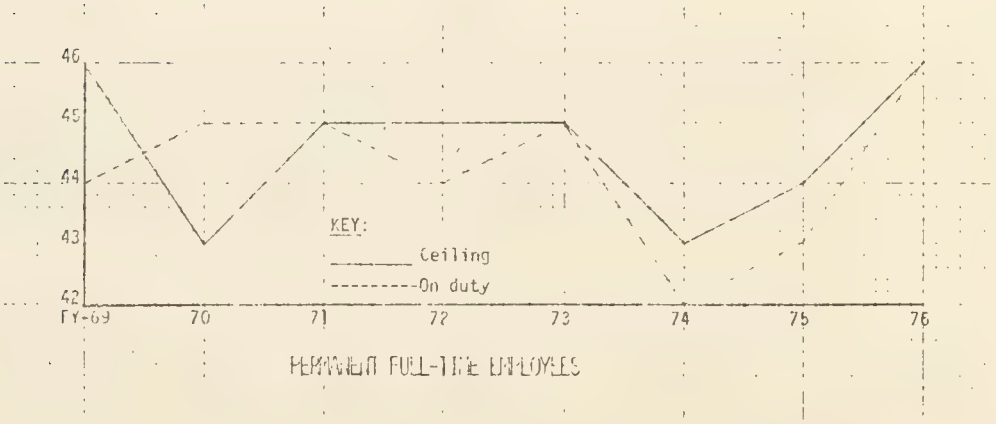
During the past year, several employees received recognition for their contributions to the operations of the program. Marguerite Kennedy, Lillian Myers, Gwen Northcutt and Minnie Kahn were awarded "Quality Increases" for the continuing high level of their performance. The staff of the Review Unit were given cash awards for superior effort in processing the National Research Service Award applications for special review in June 1974. Dr. Luz Froehlich, Assistant for Clinical Programs, was awarded the NIH Director's Award for her outstanding efforts related to the development of NIH policy on the ethics of human experimentation and in administering the activities of the Allergy and Immunology Research Committee.

Figure 4

NIAID Grants Funded and/or Reviewed -- Manpower
FY-1969 thru FY-1976



GRANTS FUNDED AND/OR REVIEWED



PERMANENT FULL-TIME EMPLOYEES

I. ALLERGY AND IMMUNOLOGY BRANCH

Activities included within the Immune System and Disease research program received the following approximate levels of support:

Type of Grant	Number	Amount
Research Grants[1/]	428	$29,893,953
Training Grants	14	972,437
Fellowships	94	1,659,455

Summary

This program is concerned with the immune system as it functions in the maintenance of health and as it is affected in immunologic, hypersensitive and allergic diseases. The program is organized into two major components.

Research efforts within the area of Allergic and Immunologic Disorders have been concerned with the dynamics of interaction between the immune system and challenging stimuli and resultant physiologic and/or pathologic effects on expression of host defenses. The scope has included studies of products and sequelae of immune reactivity evoking local or widespread effects on body tissues and systems, as well as functional impairments and deficiencies in elements of the cellular and humoral limbs of the immune system and has encompassed the entire spectrum of normal, deficient, and disordered immune responsiveness. Institute Programs on Asthma and Allergic Diseases and Pathogenesis, Prevention, Diagnosis and Treatment, as well as seven General Programs, which will be discussed later in this report under the General Program section, are grouped within this area of activity.

In the other major area fundamental studies of the Structure and Function of the Immune System are directed toward acquiring a complete understanding of immune response mechanisms at their basic cellular and molecular levels as they function in health and disease. Activities in this broadly-based program area cross traditional disciplinary lines of biology and chemistry and encompass anatomic, genetic, physiologic, pharmacologic, microbial and developmental biology and organic, physical and biological chemistry. Its scope includes studies of immunologic responsiveness in various hosts, phylogenetic studies concerned

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1/ Includes 41 Career Awards ($1,050,030)

with the development of immune response capabilities in a range of vertebrate species, ontogenic studies of immunologic function within an individual species, genetic controls of responsiveness in individual hosts, interactions of immunologically-competent cell populations within the host as well as physico-chemical studies of antigens and their homologous antibodies. Special research activities within this major program area supported by Institute Programs are concerned with Lymphocyte Biology, Cellular Immunology and Immunity, Immunochemistry, Immunogenetics and Selective Immunologic Activation.

A. Asthma and Allergic Disease Program

There is now a network of 17 Asthma and Allergic Disease Centers representing a widespread geographic distribution and concerned with research on both the fundamental and applied aspects of hypersensitivity phenomena and focused on pathophysiologic mechanisms of atopic disease and allergic manifestations. Each Center is designed to effect collaborative multifaceted approaches for development and application of methods to improve diagnosis, management and treatment of asthma and allergic disease. This area also includes two related General Programs.

Workshop:

Asthma and Allergic Disease Centers Workshop, NIH, Bethesda, Maryland, December 4-5, 1975. This endeavor was the fifth annual meeting held for Directors and staff members from the 17 Asthma and Allergic Disease Centers supported by this NIAID program. This year the work of the 100 participants centered around presentations and discussions concerned with pulmonary physiology, metabolic and pharmacologic responses of leukocytes, hypersensitivity pneumonitis and late asthma, eosinophil functions, IgE receptors and cell function and hymenoptera sensitivity.

Highlights of the Asthma and Allergic Disease Program follow:

AI-10356-03 K. F. Austen (Robert B. Brigham Hospital)

Studies have contributed to an increased understanding of eosinophil leukocyte migrations into tissue sites affected by allergic reactions. Eosinophil chemotactic factor of anaphylaxis (ECF-A) derived from tissue mast cell and basophil degranulation have been isolated and chemically

characterized as tetrapeptides of two determined amino acid sequences. By tissue culture techniques ECF-A has also been identified as a secretory product of lung tumors. Utilization of human basophil leukemic cells in biochemical studies has established that a single cell type serves as the source of the four recognized mediators of immediate hypersensitivity, i.e. ECF-A, slow reactive substances of anaphylaxis (SRS-A), histamine and platelet activating factor.

AI-12026-03 R. H. Buckley (Duke University)

Studies have demonstrated a striking incidence of polymorphonuclear or monocyte leukocyte chemotaxis abnormalities in patients with hyper IgE syndrome and with severe chronic eczema. An abnormal ability to accumulate phagocytic cells at antigenic sites may be the basic pathophysiological component in these conditions. The action of levamisole on monocyte chemotaxis suggests that its effect on immunologic function may be to stimulate the ability of these cells to be mobilized to sites of immune reactions and therefore serve as useful agents for the treatment of eczema and IgE related immune deficiency diseases.

AI-10404-06 C. E. Reed (University of Wisconsin)

Studies were directed to the elucidation of mechanisms of the pathogenesis of asthma related to the role of viral respiratory infections. In 49 patients, 3 to 60 years of age and prone to attacks of infectious related asthma triggering viruses were distributed among widely differing ages. The ability to cause asthma was not restricted to a few rhinovirus types; also included were parainfluenza and adenovirus. It was also found that antibody dependent cytotoxicity (ADC) mechanisms play a role in defense against viral infections, and that some asthmatics had a cell abnormality in the ADC capacity of mononuclear cells predisposing them to viral infections.

B. Pathogenesis, Prevention, Diagnosis and Treatment

This program activity includes the Clinical Immunology and Immunopathology Special Emphasis Program and five related General Programs.

1. Clinical Immunology and Immunopathology (SEP)

Studies within this program have been concerned with investigations of etiologic factors, pathogenetic mechanisms, detection of critical diagnostic measures, and approaches to effective prevention and control of immunologic deviations, abnormalities and dysfunctions and to develop understanding of causative factors and corresponding management of immune-related disorders of autoimmune reactivity, immune complex formation and deposition, transplantation phenomena, immune deficiency states and action of pharmacologic and chemical mediators of inflammation, immunotherapeutic modalities and immunologic mechanisms in cutaneous disorders (the newly developing area of immunodermatology). Here immunologic mechanisms of disease processes serve as the unifying factors to integrate multisystem disorders and multiorgan involvement as subjects of study.

Workshop:

Conference on Immune Mechanisms in Cutaneous Disorders, Brook Lodge, Augusta, Michigan, October 22-24, 1975. To further progress in investigation at the interface of immunology and dermatology a workshop was conducted for the introduction and exchange of data and information and generation of new ideas among research dermatologists and immunologists with complementary orientations. Forty-five participants were invited for the broad spectrum of backgrounds, experiences and viewpoints and the diverse approaches to immunology and dermatology they represented. The thrust of the conference subject matter was directed to defined disease entities where the skin could be identified as either primary target tissue or associated with other system-organ involvement in an immune process or to serve as a natural source of specific antigen in the mechanisms of immune or allergic phenomena. The design of the program centered around five major areas of concern. IgE-related cutaneous problems, immune complexes in complement and cutaneous diseases, delayed type hypersensitivity and skin diseases, cell mediated immunity and skin disease, and diseases with multiple immunologic mechanisms and responses. The work of the conference was both productive and unique in the focus of subject matter introduced, bringing together for the first time a group of people with orientations relating to a newly developing specialized interdisciplinary endeavor, immunodermatology.

Highlights of the Clinical Immunology and Immunopathology Special Emphasis Program follow:

AI-11785-03 J. M. Dwyer (Yale University)

The question of the role of T cell modulation of IgE responses has been studied. In experimental models it has been shown that IgE development seems to be independent of an ontogenetic sequence which necessarily involves the production of IgM to IgG to IgA.

A number of patients with congenital sex-linked agammaglobulinemia have been identified who have detectable IgE and allergic type reactions. Also it has been shown that the ability to be sensitized is directly related to the degree of sensitization achieved by initial immunization and that anergy of cell mediated immunity in no way affects antibody production.

In patients with primary and secondary syphilis serum was found to contain an immunosuppressive factor whereas in congenital syphilis it was found to be immunostimulatory. This may relate to depletion of T cell dependent areas in the spleens of such patients. Dichotomy in *in vitro* tests of patients with sarcoidosis and alcoholic hepatitis suggests the presence of an *in vivo* suppressive factor.

C. Lymphocyte Biology (SEP)

This Special Emphasis Program is supporting four program projects, each a multidisciplinary research center integrating the technical expertise of cell culturists, geneticists, cellular immunologists and immunochemists. Their combined efforts are designed to expand knowledge of the morphologic and functional heterogeneity of lymphocyte populations and to develop the capability for identification and selection of lymphocyte subpopulations with specific immune reactivity or antigenic composition, for hybridization of such subpopulations and for selective production of specific lymphocyte products. The goal of this program is the complete knowledge of the life history of immunocompetent cells and of the genetic and phenotypic factors that determine their fate and function *in vivo* and *in vitro*.

Highlights of the Lymphocyte Biology Special Emphasis Program follow:

AI-11378-03 G. M. Edelman (The Rockefeller University)

Studies of the maturation of the immune system in the developing animal indicate that the genes coding for the

full repertoire of antibodies are generated somatically from a small number of germ line genes during the course of development, in the absence of any strong selection with respect to antigenic specificity. Thus, the sharply restricted ability of the neonatal animal to respond to antigenic stimulation is not due to a lack of antigen-specific cells, but rather to the absence of mature cells capable of the interactions needed to sustain a full immune response.

Analysis of immune function in mature animals has been focused on the properties of antigen-specific thymus-dependent (T) lymphocytes and their distinct subpopulations which can serve as "helpers" to trigger antibody production by bone-marrow derived (B) lymphocytes and give rise to cytotoxic effector cells that destroy foreign or modified host cells. Evidence has been obtained which indicates that the H-2 antigens on the surface of the cytotoxic target cells serve as adapters to combine with tumor, viral, or other antigens to form hybrid antigens containing elements of self (H-2) and non-self. These hybrid antigens appear to serve as triggering signals for receptors on cytotoxic T cells.

Other studies concerned with stimulation of B cells by antigens and lectins have been focused on inductive pertubations of cell surfaces and subsurface microtubular components. Evidence is accumulating to indicate that these and other sub-membranous macromolecular assemblies play a key role in regulating the inductive signals for lymphocyte proliferation.

AI-11576-03 F. H. Bach (University of Wisconsin)

Genetic studies of *in vivo* and *in vitro* lymphocyte responses have demonstrated that surface molecules controlled by the leukocyte-determined (LD) and serologically-defined (SD) loci of the MHC interact and mediate the generation of cytotoxic lymphocytes. Evidence has been obtained which demonstrates that LD and SD components collaborate *in vivo* and lead to an accelerated rejection of transplanted thyroid lobes in the mouse. Similarly, an enhanced cytotoxic response has been found *in vitro* when the supernatant fluid from a mixed leukocyte culture (MLC) is added to weakly reactive cytotoxic T cells.

Studies of the MLD reaction in man have led to the development of a primed lymphocyte test (PLT) which has the potential for

a more rapid and improved matching of transplant donors and recipients and should facilitate world-wide exchange of tissues and organs for transplantation. The PLT test is a modified MLC system for typing LD antigens which many feel are more important for correct matching of transplant donor and recipient than the SD antigens. Because the test can be completed within 1 to 2 days and reference PLT cells can be prepared in advance and frozen, the development of typing trays for LD antigens, like those now available for SD antigens, appears possible. It is believed that this development not only would facilitate and improve donor-recipient matching but also would provide a tool for detailed studies and exploration of the genetic controls which the MHC exerts on human immune responses in general.

D. Cellular Immunology and Immunity

This program is focused on T- T-lymphocyte and T- B-lymphocyte interactions and their interrelationships with macrophages and other leukocytes and the cellular phenomena of antigen-processing, immunologic tolerance and enhancement. Studies in this category are based upon *in vitro* cell culture methods and *in vivo* models; the latter includes investigations on tissue origin, maturation and localization of immunologically-active cell populations. The cellular mechanisms involved in the induction, maintenance, expression and pathophysiology of delayed-type hypersensitivity immune reactivity are also included in this category. Also relevant are studies of the lymphokine and other lymphocyte mediator substances which activate macrophages and have pharmacologic effects on other leukocytic cell functions, such as chemotaxis, phagocytosis, blastogenesis, cytotoxicity, and microbial resistance.

Highlights of the Cellular Immunology and Immunity Program follow:

AI-08642-07 D. D. McGregor (Trudeau Institute)

Studies have been concerned with the mechanisms by which infected animals marshall their cellular defenses in foci of infection, especially with the production and delivery to the circulation of specifically sensitized lymphocytes and the physiological properties of these cells. In rats infected with *Listeria monocytogenes* and *Francisella tularensis*, acquired immunity can be adoptively transferred from immunized donors to normal recipients by injection of immune lymphocytes. Cytokinetic data indicate that immune lymphocytes contribute to the host's defense by encouraging

the prompt and purposeful deployment of monocyte-derived macrophages at centers of infection. These sensitized lymphocytes or their products can enhance the metabolic and microbicidal activity of macrophages; the functional activation of macrophages occurs locally at sites of microbial invasion as a result of lymphocyte-macrophage interactions.

AI-09647-06 J. M. Phillips-Quagliata (New York University Medical Center)

This research is designed to determine the functions and mechanisms of cooperation between cells in immune response and tolerance induction and the roles played by antigen, antibody, and antigen-antibody complexes. Recent efforts have been concentrated on the role of the thymus in the induction of immunologic tolerance; tolerance induction in newborn rats has been found to be thymus-dependent. Both peripheral T cells and B cells require the thymus to become tolerant; neither normal adult thymocytes, splenocytes, nor lymph node lymphocytes can replace this thymic function. Also, evidence suggests that the generation of suppressor cells, themselves able to become tolerant in the absence of thymus, requires some thymic influence.

AI-13122-01 C. S. Walters (Howard University College of Medicine)

This project places emphasis on the role of thymus-derived cells in priming and tolerance to conjugated mouse gamma globulin. Normal mice have been found to respond well to priming with this antigen but nude (thymusless) mice gave no response; the results of *in vitro* studies indicated that T cells also can be tolerized by this antigen. These data indicate that T cells are involved in priming for the immune response and in establishing immunologic tolerance.

E. Immunochemistry

Research grouped in this program category centers on chemical approaches to immunologic reactants and reactions. Studies of the chemical composition and structure of humoral and secretory immunoglobulin classes and antigens, as well as of the mechanisms and kinetics of antigen-antibody reactions, form the bulk of the activity in this area. Chemical studies of complement, its components and products, and research in

the newly-developing field of immunopharmacology which includes the synthesis, chemistry and mechanism of action of drugs, hormones, their haptenic forms, and other endogenous substances influencing or participating in the immune response also are included in this program area.

Highlights of the Immunochemistry Program follow:

AI-09652-06 J. J. Cebra (Johns Hopkins University)

Serologic and primary structural analyses of specific antibodies are being applied to assess the quality of antibodies and to understand the fine regulation of antibody quality and the molecular bases of alterations of antibody quality in immunosuppressed, unresponsive, tolerant, deviated or otherwise manipulated animals. The results of such structural studies indicate that the pattern of amino acid residues examined at "framework" sections of the variable region of heavy chain is distinctive for each antibody specificity; this finding has been interpreted to reflect the expression of a minimum number of one structural variable heavy chain gene for each specificity. A major portion of recent work has been concerned with the analysis of the variable region of heavy chains in a variety of antibodies produced in guinea pigs that were either directly immunized or manipulated in advance to alter their immune response. Primary structural analysis of antibodies from both naive and deviated animals showed that, within a group with the same antibody specificity, the same distinctive framework markers for the variable regions of heavy chains were present. When animals having T-cell unresponsiveness were used, primary structural analysis of the variable regions of their heavy chains indicated that they had a relatively intact B-cell compartment and expressed the same heavy chain variable region genes upon antigenic stimulation as were reflected in antibodies from naive animals even though they failed to show cellular immunity. Also, antibodies raised in animals rendered B-cell tolerant showed the same characteristic framework markers and sequences of variable regions in their heavy chains as antibodies from conventional animals.

AI-09684-06 A. Yaron (Weizmann Institute of Science, Rehovot, Israel)

Defined chemical modification of immunogenic synthetic amino acid polymers has provided a means to examine the

critical structural and compositional features of an antigen. In one such study, alanine was used to systematically replace the lysine residues in an immunogenic lysine octapeptide coupled to dinitrophenol. No major change in immunogenicity was noted when four alanine residues were substituted, but substitution with five or six alanine residues completely abolished the polymer's capacity to induce an immune response in guinea pigs. Similar studies have indicated a critical limitation of polymer size in induction of an antibody response; a nonapeptide polymer of lysine and alanine was found to be active while the hexapeptide was not.

AI-10580-04 J. E. Hopper (University of Chicago)

In order to define more precisely the immunoglobulin products which can be synthesized by a single lymphoid clone, studies have been performed on the myeloma proteins of patients whose sera contain more than one homogeneous paraprotein population. They have included a comparison of the idiotypic antigenic determinants (variable region determinants), N-terminal amino acid sequence analysis of the variable regions of the respective heavy and light chains, and an immunofluorescent study of the individual paraprotein biosynthesis by peripheral blood and bone marrow lymphocytes from these patients. Emphasis was given to a study of serum from a patient with Waldenstrom's macroglobulinemia which contained IgM and IgG serum paraproteins. It was noted that, in general, the paraproteins share variable heavy chain idiotypic determinants; the N-terminal sequences have shown that these regions belong to separate subgroups.

A second area of study has centered on the biological role of IgD in lymphoproliferative disease. IgD has been found to be a predominant surface immunoglobulin present on lymphocyte membranes from patients with poorly differentiated lymphocytic lymphoma.

F. Immunogenetics

This categorical area includes studies of the phylogenetic and ontogenic development of the immune response and studies of genetic controls of immune responsiveness in individual hosts, inbred strains of animals within a species, and in a variety of species. Broad comparative immunobiologic and immunochemical investigations among invertebrate and vertebrate species are appropriate subjects for study in this context. Similarly, studies of the genes and gene products of the

major histocompatibility complex and their influences on the initiation and regulation of the immune response are relevant areas of research. This program, thus, also includes the basic chemistry and biology of the immune response (Ir) and immune-associated (Ia) and other serologically-defined (SD) and leukocyte-derived (LD) loci and the surface membrane expression of these genes.

Highlights of the Immunogenetics Program follow:

AI-07757-10 H. O. McDevitt (Stanford University)

Studies are designed to identify the cellular and molecular basis of H-2 linked genetic control of immune responsiveness and immune suppression. Current experimental evidence indicates that the primary difference between mouse strains which are high and low responders to synthetic amino acid polymers is in the thymus-dependent IgG secondary response. Responder strains were found to have developed a normal T cell proliferative response to antigens under Ir gene control, while nonresponders failed to do so. This response was blocked by anti-H-2 and anti-Ia, but not by anti-Ig sera. There appears to be no intrinsic Ir gene defect in the nonresponder B cell and there is no observed restriction on collaboration between responder T cells and/or macrophages and nonresponder B cells. These results suggest that Ir genes are expressed in helper T lymphocytes and that there is no requirement for I region identity between interacting T and B cells.

AI-10781-04 L. E. Hood (California Institute of Technology)

Current studies are concerned with the multigene systems which control the synthesis of antibody and those associated with the major histocompatibility locus. The organization, expression, and evolution of the genes which control antibody synthesis are being studied by comparative amino acid sequence analysis of various antibodies. Amino acid sequences in the variable region of heavy chains from myeloma proteins have been found to correlate closely with the specificity of the parent immunoglobulins from which they were derived. Comparison of immunoglobulins from the NZB and BALB/c mouse strains indicate that different antibody-producing cells are selected for neoplastic conversion in these two mouse strains. Similarly, the N-terminal amino acid residues of variable heavy chain regions in myeloma proteins from a patient who produced both IgA and Ig myeloma proteins have been

found to be identical.

Of special interest is the development of a technique for microsequence studies of lymphocyte membrane proteins which is rapid and involves only picomole quantities of protein. Immunoprecipitation techniques were used to isolate β2 microglobulin, two D alleles and two K alleles from the H-2 locus. Partial amino acid sequence studies of these molecules by this method indicated that the D and K alleles are complex allotypes which may be coded by duplicated genes rather than by simple alleles. The development of the microsequencing technique should lead to similar analyses of other membrane molecules of immunologic significance.

G. Selective Immunologic Activation (SEP)

The objective of this Special Emphasis Program is to achieve the capability for selective manipulation of the immune response in order to purposefully direct host responses to either cell-mediated immunity or to humoral antibody production. The program supports individual research projects concerned with the nature of interactions among immunocompetent cell populations, their differential susceptibility to modifying agents and the influence of variations in the molecular features, dose and route of antigenic stimulation on functional responses of T and B cell types. The content of this program is intended to complement that of the Lymphocyte Biology Program and, as such, also supports individual projects concerned with lymphocyte recognition systems, activation, and suppression mechanisms and includes relevant studies of processes leading to lymphocyte differentiation, maturation, and their production of biologically-active substances which mediate immune reactions.

Highlights of the Selective Immunologic Activation Special Emphasis Program follow:

AI-09867-06 A. J. Crowle (University of Colorado Medical Center)

Administration of homologous antiserum to guinea pigs and mice prior to immunization has been found to prevent induction of delayed hypersensitive, cell-mediated, immune responses which mediate allograft rejection and tumor immunity. Ineffective mouse and guinea pig antisera can be made effective (contrasensitizing) by carbamylation. Both IgG_1 and IgG_2 guinea pig antibodies can be

made effective in mice; pure IgG_2 antibodies were found to be more effective than IgG_1 and IgM antibodies were ineffective. Because contrasensitization was antigenically specific, the $F(ab)_2$ fragments were expected to be the active moiety. However, neither $F(ab)_2$ nor F(ab) fragments alone at concentrations equivalent to whole antiserum or whole purified antibody was effective. These data thus suggest that Fc fragments may be essential to contrasensitization.

AI-11080-03 K. H. Kohler (University of Chicago)

Research efforts have centered on assessing the separate and combined roles of antibody to antigen and antibody to the receptors for antigen in the regulation of the immune response. When given passively to adult mice, antibody directed against the receptor for antigen was found to cause reversible and short term suppression of antibody production which lasted about as long as the passively administered antibody persisted. In contrast, anti-receptor antibody given to neonatal mice caused suppression of antibody formation which lasted for many months, apparently because the clone of antibody-producing cells had been depleted. These findings suggest that the mechanism of clonal depletion may involve antibody-dependent cell-mediated cytotoxicity. It also was found that the appearance of serum anti-receptor antibody correlated with a declining response to antigen which suggests that autogenous anti-receptor antibody may regulate the response to antigen.

AI-12908-02 A. Nisonoff (Brandeis University)

The mechanism by which antiidiotypic antibodies can suppress the appearance of the corresponding idiotype within an inbred strain of mouse is being examined. Using the techniques of adoptive transfer, lymphocytes from suppressed animals have been found to be responsible for idiotype suppression. These cells are believed to act either directly by competing for antigen with normal cells or by inducing the formation of other idiotype-specific suppressor T cells in the recipient animal. These findings suggest that suppression of particular idiotypic specificities may provide an effective approach to the elimination of a specific immune response without a generalized depression of immunity.

H. General Program

There are two general programs within the Asthma and Allergic Disease Program area:

1. Allergic Diseases and Immediate Hypersensitivity

Within this category the basic and clinical aspects of allergy and allergic diseases and all fundamental facets of reaginic or IgE-mediated hypersensitivity are being considered. Investigative studies have been directed to the atopic diseases, anaphylactic reactivity, IgE mechanisms, chemical mediators of immediate hypersensitivity, pathogenesis of allergic reactivity and specific immunotherapy.

AI-09529-07 H. F. Dvorak (Massachusetts General Hospital)

A lymphocyte product chemotactic for basophils has been found and provides an explanation for the mechanism by which granulocytes may be attracted to sites of cutaneous hypersensitivity reactions. The finding that interaction between basophils and specific antigen inhibits their subsequent migration in response to a chemotactic stimulus may explain why antibody can turn off these types of cutaneous reactions. An important development has been the successful culture of purified basophils for several days allowing for studies to elucidate this cell's behavior in both immediate and delayed types of immune activity, co-culture studies with tumor cells and other target antigens, and studies on contents and synthetic rates of pharmacologic agents in cell granules.

AI-12148-03 I. L. Bernstein (University of Cincinnati)

Investigations have been concerned with platelet dysfunction and catecholamine excretion in immunologic and non-immunologic asthma. Those with immunologic asthma may demonstrate platelet aggregation abnormalities consistent with an aspirin-like thrombopathy. Such aberrations are more likely to occur in patients with increased levels of serum IgE. Concentration of this immunoglobulin could explain occasional platelet dysfunction in ostensibly normal persons with unsuspected, minor allergic symptoms. The association of asthma with platelet abnormalities offers leads to investigate

platelet physiology, aspirin intolerance and human platelet activator factor.

AI-07290-10 L. M. Lichtenstein (Johns Hopkins University)

Release of eosinophil chemotactic factor (ECF) from all human granulocytes has been determined suggesting that it is a product characteristic of inflammation, and that, as a result of phagocytic processes, an inhibitor of its activity is released. By cell stripping techniques the number of IgE molecules per basophil has been enumerated and found to range between 5,000 to 500,000 so that correlation with histamine release phenomena can now be feasibly approached.

AI-11632-02 C. D. May (National Jewish Hospital, Denver)

Findings of this clinical investigation indicate that clinical history is unreliable as to adverse reactions to food. Positive skin tests, however, are closely related with possible double blind food provocation tests. Symptoms evoked to food allergens, even in asthmatics, are primarily gastrointestinal and urinary excretion of histamine analogs is not increased during immediate immunologic hypersensitivity reactions to these foods. The indication is that reaction is chiefly local and restricted to the gastrointestinal tract. The related finding of high control in in vitro tests for antigenic release of histamine from peripheral leukocytes is sufficiently characteristic of subjects who react to foods to constitute a sign of clinically significant immediate hypersensitivity.

2. Asthma

Programs within this area have been dealing with problems in allergic asthma, reversible bronchospastic syndromes of non-extrinsic demonstrable etiologies and entities in which infectious, sympathetic nervous system (adrenergic, cholinergic) endocrine or inflammatory processes may play primary, predisposing or contributory roles. Representative areas under study include reversible bronchospastic asthmatic syndromes, bronchoprovocation challenge and pulmonary function tests to evaluate inhalant materials, allergens, chemical mediators, adrenergic agonists and antagonists, physical factors and pharmacologic agents and sensitization mechanisms in infection and pulmonary defense.

AI-11759-03 R. Patterson (Northwestern University)

In the study of developed primate models of asthma it was found that response to antigen is not vagaly mediated but that vagal tone may modulate responsiveness. Since atropine does not affect histamine or prostaglandin induced changes in pulmonary function there are probably specific receptors for these two agonists also acting without vagal mediation. Related studies were designed to determine the sensitivity of pulmonary function parameters for various purposes. In these it was found that closing volume and closing capacity are effective tests to screen for chronic obstructive pulmonary disease in the human since they will be unaffected by individual differences in vagal tone as found in asthmatics. The associated administration of influenza virus vaccine given by nasal spray while not affecting normal individuals resulted in clinical illness and some increase in airway obstruction within three weeks after administration to patients with chronic obstructive lung disease.

The use of polymaerized ragweed antigen shows signs of improving the efficacy of immunotherapy. High doses were attainable in less than ten injections without incidence of untoward reactions and with marked increase in blocking antibody, lower rise in seasonal IgE and diminished histamine release values.

There are five general programs within the Asthma and Allergic Disease Program.

1. Delayed Hypersensitivity Disorders

This component has focused on basic mechanisms of cell-mediated immune reactivity, their in vivo expressions, and human diseases resulting from delayed hypersensitivity processes. Programs in this area now include clinical investigations on etiology, pathogenesis and therapy of allergic contact dermatitis, drug reaction syndromes, delayed hypersensitivity manifestations of infectious diseases, lymphoproliferative-granulomatous diseases of undetermined etiology (e.g. sarcoidosis, Hodgkin's disease, Crohn's disease, Wegener's granulomatosis) and granulomata associated with chemical sensitization.

2. Immunodeficiency

This program has been concerned with etiology, pathologic effects, prevention and treatment of structural and functional deficiencies of the immune system involving both naturally-occurring and acquired states. Ongoing studies have focused upon congenital absence, failure of development or other disorders affecting thymic or bone marrow cellular elements, deficient production, abnormal loss or catabolism of immunoglobulins, deficiencies of specific complement components, and defective host defenses due to abnormalities of leukocytes.

AI-10343-05 R. Hirschhorn (New York University)

Studies have been concerned with adenosine diaminase (ADA) deficiency disorders, especially those with autosomally inherited severe combined forms. Two specific isoenzymes have been identified in tissues of normal humans and patients. Prenatal diagnosis of severe combined immunodeficiency disease (SCID) associated with ADA deficiencies have been made possible leading to successful bone marrow transplantation treatment. Characterization of residual enzyme activity in fibroblasts give evidence for a mutant enzyme indicating that SCID is due to a mutation at the adenosine diaminase locus. Experimental data further indicate that ADA deficiency is causally related to cellular immune defects and suggest a possible role for enzyme replacement in the therapy of this disorder.

3. Inflammation

Studies have been directed to areas concerned with inflammatory inducing components, circumstances and sequelae of immune reactions. Ongoing investigative programs deal with activation of the complement cascade and liberation of anaphylotoxins and chemotactic complement fragments resulting in vascular permeability, blood clotting, leukocytic infiltrations, fibrinolysis and vascular endothelial damage. Other related problems addressed have included cellular biologic processes, chemistry and pathophysiologic actions of leukocyte lysosomal enzymes and mechanisms of their release, and cytotoxic, chemotactic and cellular immobilizing secretory products of lymphocytes employed in host defense pathways.

AI-07007-11 F. J. Dixon (Scripps Research Institute)

Experimental data from this program project have contributed information to several areas of immunologic and inflammation research. For an understanding of immunopathologic mechanisms responsible for human glomerulonephritis, immunochemical and immunohistologic technology capable of providing pathogenetically based diagnosis and prognosis of this disease have been developed. Immunologic responsiveness, not tolerance, to viruses introduced in utero or at time of birth have been demonstrated. By use of RaJi cells a sensitive, specific and quantitative technique for the measurement of these circulating immune complexes has been developed. In related studies the immunopathogenicity of an oncorna virus inducing antinuclear antibody and immune complex glomerulonephritis in experimental models has been demonstrated.

4. Transplantation

Studies within this program have been concerned with transplantation mechanisms and immunogenetics employing in vitro and in vivo studies of animal models and human systems. In addition to transplantation phenomena, investigation has centered upon reconstitution of immunodeficient patients with donor bone marrow, fetal thymus or fetal liver tissues. Research in ongoing studies is dealing with the immunogenetics of tissue antigen matching, transplant survival, prevention, control and treatment of graft-versus-host disease, pharmacologic agents and biologic methods for effecting immune tolerance and enhancement of graft survival, and the immunologic factors responsible for disease processes recurring in newly-transplanted donor tissue and the emergence of immunologic diseases initiated by the transplanted organs.

AI-12754-02 R. L. Simmons (University of Minnesota)

Studies were directed to define mechanisms whereby the immunogenicity of organ allografts may be reduced by pretreatment of the graft thereby permitting survival. In dog models it was found that the systemic immunosuppressive effects of soluble concanavalin A (Con A) cannot explain the prolonged tolerance of Con A perfused kidneys. The effect instead was that of antigens being modified by Con A with induction of a degree of partial hypoactivity in the host permitting

the survival of unmodified grafts. Data were consistent with the hypothesis that antigen modification may supplement immunosuppression in the prolongation of renal grafts. In non-specificity of changes induced by a great variety of drugs including heparin, dextran and dipyridamole it is suggested that an altered donor cell membrane is a crucial alteration in the modification of graft immunogenicity.

AI-10088-06 R. Walford (University of California, L.A.)

The frequency and, in some cases family genetic analysis, of HL-A factors in several disease states have been studied. Statistically significant disequilibrium in systemic lupus erythematosus, myasthenia gravis, multiple sclerosis, acute lymphatic leukemia and Hodgkin's disease has been identified. On the other hand, there is no clear-cut suggestion of disequilibrium in chronic lymphatic leukemia, multiple myeloma, juvenile diabetes and leprosy. In multiple sclerosis relation of HL-A-A7 to the level of hypersensitivity response to human brain antigens was also noted. In this situation the spontaneous occurrence of antibrain antibodies was also noted.

AI-10921-05 J. R. David (Robert B. Brigham Hospital, Boston)

Absence of an immunologic blocking factor in the serum of women who chronically abort has been identified. The finding that this factor, capable of preventing an effector response by sensitized lymphocytes, is detectable in the sera of women able to maintain a term pregnancy but absent from sera of habitual aborters suggests that it may be necessary for the successful maintenance of fetal allografts.

AI-11729-03 R. E. Rocklin (Robert B. Brigham Hospital, Boston)

The biologic significance of a factor present in the plasma of multigravid women which specifically blocks a lymphokine response (MIF) by maternal lymphocytes to paternal antigens was investigated. Studies indicated that some women who chronically abort have lymphocytes sensitized to paternal antigens and an absence of the specific blocking activity in their sera.

This suggests that the inability to prevent MIF production against paternal lymphocytes may be important in maintaining normal term pregnancies.

5. Immunological Diseases; Immune Complex and Autoimmune Disorders

Studies within this category include studies of disorders arising from the generation of monoclonal immunoglobulins, formation and deposition of antigen-antibody complexes, the development of autoimmune states and research focused upon the basic immunobiologic, immunochemical and immunopathologic mechanisms involving connective tissue, skin, cellular elements of the blood and hematopoietic organs, bronchopulmonary, cardiovascular, renal and gastrointestinal tracts, hepatic, splenic, endocrine glandular and osseous tissue and nervous system elements in single-organ or multisystem character. Some of these projects are focusing on immune complex mechanisms, dynamics of antigen-antibody reactions, chemical manifestations of Arthus' reaction and serum-sickness type hypersensitivities, pathologic processes effected by abnormal serum proteins, basic and clinical aspects of autoimmune phenomena and immunopathology and methods of prevention and control of autoimmune disease.

AI-06477-13 T. C. McGuire (Washington State University)

The study of experimental models in mink and ferrets infected with aleutian disease (AD) virus has contributed information to our understanding of the immunopathology of glomerulonephritis and vasculitis. Animals maintaining only low levels of replicating virus and circulating antigen form fewer inflammatory producing virus-antibody complexes. Those with larger amounts give evidence of arteritis, glomerulitis, hepatic necrosis and bile duct hyperplasia. Lymphocyte function studies have also demonstrated a suppression of T cell responsiveness which may contribute to viral persistence. The increase in B cells, some of which secrete antibody directed against the AD virus, suggests that plasma cell infiltrates result from expansion of clones of B cells which are specific for the viral antigens.

K04 AI-70968-03 V. Agnello (Tufts University)

Two new sensitive radioimmunoassays have been developed

for detection of complement fixing and non-complement fixing immune complexes. The availability of these techniques can now be applied to important points for study in patients with systemic lupus erythematosus, rheumatoid arthritis, infectious hepatitis, chronic active hepatitis, idiopathic glomerulonephritis and melanoma. The close linkage of C2 deficiency gene with the HL-A 10, B 18 haplotype has been confirmed in situations characterized by atypical lesions of SLE. Long term serial studies of SLE patients have provided evidence for the involvement of DNA-anti-DNA complexes in subendothelial deposits in SLE nephritis.

AI-10334-06 G. A. Andres (SUNY-Buffalo)

The study of immune complex phenomena in experimental animals provides an explanation for splenic pathology and splenomegaly in patients with underlying immune complex disease and an approach for further investigation of the relationship between circulating complexes and idiopathic pulmonary fibrosis.

Studies on immune complex disease in man have shown that immune deposits may localize in the spleen of patients with acute post streptococcal glomerulonephritis. Also, identification of deposits of IgM, heterophil antibodies and antigens and C3 in the mesangium give evidence for the interpretation that heterophile antigen-antibody complexes may well play a role in the pathogenesis of infectious mononucleosis. The demonstration of immune deposits and polymorphonuclear neutrophils in mesothelial vessels now helps to explain why pericarditis, pleurisy and peritonitis are among the most common of symptoms in systemic lupus erythematosus.

AI-11843-04 R. A. Good (Sloan-Kettering Institute)

The issue of measles antibodies in cerebrospinal fluid and serum was restudied using a sensitive radioimmuno-assay. It was possible to demonstrate measles antigens towards cerebral spinal fluid (CSF) and to detect serum humoral response. Evidence suggests that different structural components of the measles virus may act as antigens. From a study of patients with multiple sclerosis and related neurologic disorders it is suggested that IgG antibody directed towards measles may be present in CSF but not in serum.

AI-10931-05 H. G. Bluestein (University of California, San Diego)

Ability to suppress specific responses with antisera directed at cell membrane antigens provided a useful probe of the linkage between antigen-receptor interactions and biochemical events occurring during lymphocyte activation. The demonstration that specific immune responses can be blocked with anti-histocompatibility reagents suggests the possibility for further development of methods to produce specific immunosuppression in patients with immune mediated diseases without suppressing beneficial immune responses.

II. BACTERIAL AND MYCOTIC DISEASES BRANCH

The approximate level of research support in bacterial and mycotic diseases:

Type of Grant	Number	Amount
Research Grants 1/	201	$10,710,208
Training Grants	15	944,646
Fellowships	48	879,612

Summary

Advances in infectious diseases research, leading ultimately to medical application to alleviate specific health problems, are closely intertwined with advances in fundamental knowledge developed in the basic sciences. Support of research in the basic disciplines of cell biology, physiology, microbiology, and immunology are the fundamental areas where newer knowledge will have its greatest impact on the infectious diseases that affect the health of the population. It is a well-known truism, but one worth emphasizing, that support of fundamental research in these basic sciences must be maintained. The Bacterial and Mycotic Diseases Branch continues to support many worthwhile projects in fundamental research that have a direct bearing on infectious diseases of bacterial or fungal origin.

The more targeted research areas, identified as Special Emphasis Programs, have shown continued growth - this is especially true of the program in Sexually-Transmitted Diseases (STD), which should reach its highest level since adoption of this program by the National Advisory Allergy and Infectious Diseases Council. The program on Hospital-Associated Infections (HAI) is also continuing to grow, but at a more modest rate. This problem area, although a major source of excess hospitalization costs and patient morbidity, is more difficult to expand and develop and to stimulate interest in among the biomedical community. It may be that it offers less of an intellectual challenge and stimulus than does research in the more categorized problem of STD. Future programming efforts, nevertheless, will be intensified in this problem area.

The Special Emphasis Program on Streptococcal Diseases and Sequelae

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1/ Includes 9 Career Awards ($230,141)

(STP) is relatively static. There does not seem to be an overwhelming desire, with some exceptions, on the part of new young investigators to be trained in or to do research in this field. The exception seems to be a rekindling of interest in research on the B group of streptococci. Greater programming efforts will also be devoted to expansion of this area as a worthwhile research endeavor. It should be noted that the Dollar Allocation by Program Area (DAPA) mechanism has served a useful purpose in developing these programs.

A. Biology of Sexually-Transmitted Diseases (STD) (formerly Venereal Diseases) (SEP)

This problem continues as a major public health area of concern; gonorrhea is the most common infection reportable to the Center for Disease Control. Although research has led to significant advances in our understanding of the causal organism and of the disease, the number of cases continues to increase. It is obvious that this is a behavioral and social, as well as a medical problem, so that different control and educational strategies may have to be developed to aid in the ultimate containment of the epidemic. Non-Gonococcal Urethritis (NGU) currently is identified as perhaps the second most common of the sexually-transmitted diseases. A mounting research effort is now underway to develop the knowledge that will be necessary for its control. The question of the major causal organism, considered by many to be Chlamydia trachomatis, has not been clearly defined. The B group of streptococci, also identified as a possible venereal disease, remains to be clarified as to the meaning and dimensions of the problem. Recent reports implicate hepatitis B as a venereal disease, particularly in closed homosexual populations; likewise, infections with Entamoeba histolytica have also been involved in a similar population.

The STD program, as of the end of this fiscal year, will reach a total funding level of about $3.25 million. Included in this support will be four program projects, or Venereal Disease Research Centers; a total of 28 Fellows are also undergoing postdoctoral training in various aspects of venereal disease research.

Meetings:

The First Symposium on the Biology of the Parasitic Spirochetes, supported by the NIAID, was held at the University of Minnesota in June of 1975. This meeting attracted a wide audience and was acclaimed as a most successful exposition of the state of our knowledge on the treponemes and the other spirochetes causing human diseases. Publication, as a book by Academic Press, is expected momentarily.

A Workshop on Non-Gonococcal Urethritis and Related Oculogenital Infections was held at the W. Alton Jones Cell Science Center, Lake Placid, New York, on April 28-30, 1976. This meeting, organized and supported by NIAID, drew together the outstanding experts on the subject for an in-depth discussion of the entire problem. Reports were presented by workers from Great Britain, Denmark, Sweden and Canada, as well as the experts from all over the United States. Discussions centered on the biology of the causal organisms (Chlamydia trachomatis and Ureaplasma), the epidemiology and pathogenesis of the disease syndrome in males and females, therapeutic measures, and future trends for research on this problem.

Publication of the proceedings is expected during FY 1977.

Some important research results in the Special Emphasis Program on Biology of Sexually-Transmitted Diseases are reported here.

PO1-AI-12192-02 K. K. Holmes (University of Washington)

A number of developments from this productive research group will have considerable bearing on our understanding of several of the sexually-transmitted diseases (STD), especially non-gonococcal urethritis (NGU). The role of the chlamydial agent in NGU has been demonstrated - this agent has long been believed, particularly by workers in England, to be the sole cause of NGU. Additionally, evidence by Holmes and his group was obtained that Ureaplasmas (T-strain Mycoplasmas) are associated with chlamydia-negative cases of NGU. Other studies, on gonorrhea, showed that a virulent strain of gonococci, associated with disseminated infection, could be characterized on the basis of auxotrophic nutritional requirements for three amino acids, arginine, hypoxanthine, and uracil (AHU). Examination of over 500 isolates showed that the prevalence of AHU strains isolated from uncomplicated gonococcal infection varied markedly throughout the United States. This may explain regional differences in the occurrence of disseminated gonococcal infection. These gonococci were further shown to be uniquely resistant to the complement-dependent killing by normal human serum.

PO1-AI-12618-01 J. M. Knox (Baylor College of Medicine)

Double-blind clinical studies on treatment of herpes simplex (HSV-2) genital infections by dye-light photoinactivation are continuing, with promising results. This treatment method is, however, controversial both as to its effectiveness

and its safety. Nevertheless, a careful, well-controlled trial, such as in this project, is necessary to determine if the method is of true clinical value. Data to date indicate that, if treatment is begun early, the method has significant value, and also that recurrences are less severe and show a longer delay between each bout of infection. In other studies, the major envelope glycoprotein complex of HSV-2 has been isolated from the infected cells and purified. Antisera to this purified glycoprotein were produced in rabbits and these sera were shown to neutralize HSV-2 but not HSV-1 infections. This material could have the potential to induce immune protection against active HSV-2 infection.

PO1-AI-12381-02 R. L. Nichols (Harvard University)

One of the subprojects in this program is a study of a naturally-occurring chlamydial infection in guinea pigs that is sexually transmitted. This is a useful model for clarification of several features of the human chlamydial venereal disease. A standard chlamydial agent, called gpic, was shown to cause resistance to reinfection by urethral challenge in the male guinea pig urethra after recovery from an initial sexually-transmitted infection. Evidence has been obtained that the induced immunity is generalized as well as local. Male to female sexual transmission of this agent has been well documented - the agent's characteristics are similar to the human infecting agent, thus much can be learned from this animal model system. In studies on cytomegalovirus (CMV), a highly-specific, indirect fluorescent test (IFA) was developed; a specific fluorescence confined to the viral intranuclear inclusion material is produced. This test should be useful in distinguishing new human CMV isolates from other human herpes viruses. Urethral secretions from male patients at a venereal disease clinic showed the presence of specific CMV antibody; CMV antibody was also seen in the sera of these patients, and in cervical secretions of female venereal disease patients.

AI-10615-05 E. C. Gotschlich (Rockefeller University)

The protein composition of the outer membrane of the gonococcus (GC) was found to be similar to other Gram-negative organisms. A single major membrane protein antigen was prepared; these protein extracts from a number of different GC strains were found to segregate into several well-defined classes. Antisera prepared to different major membrane proteins could be used to type a number of different gonococcal strains obtained from different areas in the

United States and abroad. This serotyping system appears to be stable in that a large number of these strains could be typed and retyped on different occasions and in more than one colonial form, always giving identical results. This serotyping system has considerable public health significance and may prove to be very useful in epidemiologic studies.

AI-12469-02 D. J. Drutz (University of Texas)

A new animal model has been developed for studying gonococcemia; this model, reliably produced in rabbits, represents a highly-significant advance. Rabbits were infected by passing polyethylene catheters across the aortic valves, followed by injection of up to $1x10^8$ gonococci of the virulent T-1 colony type. Penicillin-resistant gonococcal strains failed to elicit evidence of infection, as did injection of gonococcal colony type T-3 (avirulent strain). A striking accompaniment of gonococcal endocarditis was the presence of gonococcal hepatitis - cultures of hepatic lesions were filled with gonococci. These data support published observations suggesting a relationship between gonococcal penicillin susceptibility and capacity for production of hematogenous infection.

AI-11643-02 A. I. Braude (University of California, San Diego)

In these studies on gonococcal infection, the Principal Investigator has made the significant discovery that there is a relative serum resistance of virulent gonococcal colonial types 1 and 2; there is also no difference in number of binding sites or avidity for antibody between the avirulent and virulent types. This is a newly-reported observation that resistance to killing by antibody and complement contributes to gonococcal virulence. This finding opens the way for studying the mechanism of gonococcal virulence through an analysis of factors causing resistance to antibody and complement. Other data also show that there is a distinct trend toward poorer killing of the gonococci by serum factors just before menstruation - this fits with the observation that there is an increased susceptibility to gonococcal septicemia during menstruation.

AI-11278-03 R. A. Finkelstein (University of Texas)

This investigator has been studying growth and pathogenicity of the gonococcus in a chick-embryo model system. He has reported that iron compounds added to relatively avirulent types of gonococci increased their virulence almost 100-fold.

Iron-binding compounds (chelates) significantly reduced the gonococcal infectious capability of the virulent types 1 and 2 gonococci. The Principal Investigator has also presented a theory that the pili of the types 1 and 2 gonococci may be involved in iron transport or iron-binding, which may have a direct effect on the virulence. Other investigators have shown that iron causes increased numbers of the gonococci to adhere to human sperm cells; this could therefore be a significant factor in increase of virulence. The availability of iron, the Principal Investigator believes, could possibly have an effect on the severity of the disease symptoms.

AI-10107-05 E. Juni (University of Michigan)

A simple transformation assay system for identification of N. gonorrhoeae present in clinical materials is under development by this investigator. A mutant of the gonococcus that is transformed back to the wild phenotype by DNA from all strains of N. gonorrhoeae forms the basis of the test system. The mutant is unusual in that no interaction with DNAs from nongonorrheal Neisseria species occurs. The test system seems simple, accurate, and cheap; it is possible to detect the gonococcus using only organisms present on a urethral or vaginal swab, with the test being completed in less than 24 hours. This transformation assay is also being developed to detect the presence of Moraxella urethralis and Acinetobacter species. The Acinetobacter are opportunistic pathogens causing considerable morbidity and mortality in immune compromised patients. These tests are currently being used in several clinical laboratories.

AI-11283-04 J. B. Baseman (University of North Carolina)

Using radioassays, this investigator demonstrated the ability of virulent Treponema pallidum to synthesize protein and to degrade glucose to CO_2 and specific end products. He has also shown the dependence of T. pallidum metabolism on O_2, which serves as a stimulant of protein synthesis, motility, and substrate degradation. Motility of the virulent organism is vigorous in presence of O_2, and sluggish in its absence; resting suspensions also require O_2.

These findings bear out the earlier discovery by C. D. Cox (University of Massachusetts) that T. pallidum has an aerobic metabolism.

These advances in our knowledge of this organism are important as major events that may lead to eventual in vitro cultivation of this organism.

AI-08124-09 R. C. Johnson (University of Minnesota)

This project centers on comparative studies of parasitic spirochetes, particularly Treponema pallidum. A maintenance medium developed for retention of the motility of T. pallidum for storage in vitro also maintained the full virulence of the organisms, but only for a short time. Virulence decreased rapidly until, by day 5, only avirulent treponemes emerged. BCG cells were injected into rabbits to enhance nonspecifically their cell-mediated immunity; the animals were subsequently challenged with virulent T. pallidum. The BCG treatment did not enhance resistance of the rabbits to syphilitic infection; in fact, a significant increase in the number of lesions per rabbit occurred after intravenous challenge. Immune serum given to BCG-treated rabbits resulted in an essentially normal development of syphilitic lesions.

In an attempt to examine other animals as a potential model system, normal piglets were kept at 16°C and gnotobiotic piglets were kept at 32°C; these animals rapidly destroyed injected virulent T. pallidum.

Gnotobiotic piglets, however, kept at 26°C showed slightly slower clearance of the organisms. It is concluded that the high body temperature of the piglets had an important influence on the survival of T. pallidum.

B. Hospital-Associated Infections (HAI) (SEP)

The public health problem of nosocomial, or hospital-associated, infections continues to be a very worrisome one; the numbers of deaths, and the high costs of excess hospitalization resulting from these infections, are difficult to determine exactly, but they are known to be very high. The populations most at risk are those patients either with impaired immune defenses or undergoing cytotoxic therapy for malignancies or renal transplants. Other patients at high risk are those with burn wounds, and surgery patients. The morbidity and mortality rates of these classes of patients are significantly high and show no signs of diminishing. This Special Emphasis Program, because it deals with a difficult, multifaceted problem, is consequently broad-ranging and not as neatly categorized as are other Special Emphasis Programs of the NIAID. The program, nevertheless, is continuing to grow but has yet to reach a critical mass that will generate significant enthusiasm in the biomedical community. Despite this, a number of highly-qualified and recognized experts have made interesting observations and obtained research data that may well have a very great impact on this serious problem. The program currently includes 11 postdoctoral Fellowships among the 48 funded awards; total expenditures are now at approximately $1.78 million.

Some of the important research results by grantees in this program are described here.

AI-10108-06 A. I. Braude (University of California, San Diego)

One of the most intriguing new developments, which will undoubtedly have a significant impact on the therapy of serious and life-threatening bacteremias incurred in some hospital-associated infections, is the work described by Dr. Braude. In the current progress report, studies were continued on serum therapy of patients suffering from Gram-negative bacteremia, with an antiserum obtained from human volunteers inoculated with the J5 vaccine. This vaccine, reported on previously, is based on the mutational core lipopolysaccharide (LPS) obtained from a specific *E. coli* strain O:111. It was also reported earlier that this antigen showed wide cross-reactivity against core LPS of all clinically-important causes of fatal Gram-negative bacteremias. Serum therapy given to a large number of patients with severe Gram-negative bacteremia resulted in a high survival rate, and thus showed that a strong

potential existed for successful therapy with the J5 human antiserum. This antiserum also caused a dramatic increase in survival of rabbits from experimental Pseudomonas septicemia, even though there are great biological differences between Ps. aeruginosa and E. coli 0:111. Further clinical double-blind studies on the J5 antiserum are continuing.

AI-06931-10 P. G. Quie (University of Minnesota)

The thrust of this proposal is to understand some of the factors affecting immune responses to infecting agents in immune-compromised patients. Chemiluminescence (CL), as a response to the burst of oxidative metabolism occurring in phagocytosis, was developed as an indicator or assay for opsonization. An extremely close correlation was found between the kinetics of intracellular killing and production of CL. Phagocytosis of bacteria and CL responses of human neutrophils are closely interrelated; measurement of CL appears, therefore, to be a reliable assay for measuring opsonic activity of serum as well as the metabolic response of phagocytes. Human monocytes produced only one-third of the CL response of neutrophils; this diminished CL response is consistent with the slower phagocytosis of particles by these immature cells. The major part of the CL occurs as a consequence of superoxide production during phagocytosis. The CL response of leukocytes is used routinely in the Principal Investigator's laboratory for rapid evaluation of phagocytic cells of patients suspected of having chronic granulomatous disease. Additionally, patients on chronic hemodialysis are being studied because they show a high mortality rate from bacterial infection. Neutrophil chemotactic responsiveness in such patients was shown to be depressed. Hemodialysis impairs neutrophil function and these results suggest that this neutrophil dysfunction may be related to the increased risk of infection in uremic patients.

AI-12402-02 P. G. Quie (University of Minnesota)

A major clinical problem of great concern is serious systemic infection with the pathogenic fungus Cryptococcus neoformans. Cryptococcal polysaccharide, extracted from cultures, was used to immunize rabbits and the IgG was then separated from the rabbit antisera. Using these reagents, as little as 25 nanograms of cryptococcal polysaccharide could be detected in cerebrospinal fluid (CSF) or urine, with the

concentrated rabbit IgG. The reaction, furthermore, was specific for cryptococcal infection. Counterimmune electrophoresis (CIE), using the anticryptococcal polysaccharide IgG, provided rapid diagnosis of cryptococcal disease in a number of patients; response to therapy could be followed by reduction in quantity of cryptococcal antigen in the CSF. The technique of CIE, as shown in these studies, has proven to be clinically-useful for detection and diagnosis of cryptococcal infection.

AI-08504-07 P. F. Bartell (College of Medicine and Dentistry of New Jersey)

The goal of this project is an investigation of the extracellular slime layer of Pseudomonas aeruginosa and its role in disease. This slime layer produced by the microorganisms may be involved, in part, in the pathogenesis of cystic fibrosis. Results show that, in a mouse model, intraperitoneal injection of the extracellular slime (a polysaccharide containing glycolipoprotein) from the microorganisms caused a leukopenia followed by death of the mice. Mice surviving lethal challenge overcame the leukopenia and released large numbers of neutrophils into the circulation. Leukocytes of mice injected with the slime glycolipoprotein were agglutinated by antiglycolipo-protein serum, indicating an association between the leukocytes and the glycolipoprotein. Results in the mouse model indicate a rapid association of the bacterial slime glycolipoprotein with the peripheral leukocytes, beginning about five minutes after infection. The liver appeared to be a target organ for sequestering the neutrophil-glycolipoprotein complex, thus accounting possibly for the leukopenia observed in the mice. The bacterial cell product appears to playa rolein the infectious process by this complicated organism.

AI-05283-13 P. V. Liu (University of Louisville)

This study has concentrated on purification and characterization of the toxins of Pseudomonas aeruginosa. The Principal Investigator has reported at least three different types of exotoxins - the exotoxin A functions in a manner very similar to the diphtheria toxin. Both toxins act as enzymes; the Pseudomonas exotoxin A functions as an enzyme without modification, whereas the diphtheria toxin has an active

fragment that can be split off the toxin molecule. Two other exotoxins, B and C, produced by this organism are, however, destroyed in culture by proteolytic enzymes produced by the micro-organism. Attempts are underway to search for nonproteolytic strains.

The lecithinase of Ps. aeruginosa is not involved in pathogenesis of skin infections, but may be an important factor in lung infections by this organism. The main component of the lung surfactant is a lecithin, which may be destroyed or altered by the Pseudomonas lung infections. It was shown that bronchial washings containing the surfactant supported production of a significant amount of the bacterial lecithinase.

Efforts to establish an international standard for serotyping of Ps. aeruginosa, based on the heat stable somatic antigens, is progressing satisfactorily. So far, 17 distinct serotypes have been identified; these will form the nucleus of a future international scheme for serotyping of these important microorganisms.

AI-12145-02 R. D. Diamond (Michael Reese Hospital, Chicago)

Studies were performed on the antibody-dependent killing of cryptococci; killing did not occur unless cell-to-cell contact (fungus-leukocyte) was assured. Results indicate that multiple types of peripheral blood leukocytes, excluding T-cells, are capable of antibody-dependent fungicidal activity. It was also found, by electron microscopic study, that non-phagocytic killing of cryptococci occurred by monocytes, granulocytes, and small lymphocytes. A guinea pig model of infection was developed - these animals showed delayed-type hypersensitivity to cryptococci as early as seven days. Preliminary studies also suggest prolonged survival of animals immunized with killed cryptococci in Freund's adjuvant. These studies should help to clarify our understanding of different cellular host defense mechanisms and their importance in overcoming opportunistic mycotic infections.

C. Methods for Other Diseases

1. Streptococcal Diseases and Sequelae (STR) (SEP)

There is an increasing awareness that the B group of the streptococci are a major problem in causation of serious

infections, particularly in the newborn. The meningitis infections and septicemias play a significant role in death or serious damage in the nursery population, identified as the major at-risk group to the streptococcal B infections. A recent workshop co-sponsored by the NICHD and NIAID explored this problem in depth. The recommendations made were that more attention be paid to B group streptococcal infections in order to enlarge our knowledge of this disease entity and hopefully to develop methods to eliminate it from the newborn nursery. The role of the group B streptococcal microorganisms in relation to venereal carriage is also not clear, although it has been found in about 18% of sexually-inexperienced females; this rate shows a rise to about 35% colonization, depending on the level of sexual experience. The role of the male sex partner has not been clearly defined in the spread of this organism. The problem of acute glomerulonephritis and rheumatic valvular heart disease, as the major serious sequelae of the group A streptococcal infections, remains as one still deserving of continued attention. This Special Emphasis Program has reached a rather static stage; for example, no specific training of Fellowship support was sought from the NIAID during this past fiscal year. This is an indication of a seeming lack of interest in this research area by the new young investigators who are just beginning their research careers. This program as currently supported now comprises 15 awards for a total of $960,128.

Some recent findings of interest in this research area are described here.

AI-11161-05 C. Panos (Thomas Jefferson University)

This investigator has reported the first instance of the rapid adaptation of a stabilized, though osmotically-fragile, L-form of a human pathogen (group A streptococci) to grow in physiologically-isotonic media. Of even greater interest is the demonstration that these supposedly innocuous microbial forms will rapidly destroy not only human heart cells in tissue culture, but human kidney and liver cells as well. These results increase speculation of the involvement of bacterial L-forms in pathogenesis and in hyperimmune responses. It was further shown that the stable L-forms of Streptococcus pyogenes could survive in immunosuppressed mice. After peritoneal inoculation the L-forms were consistently recovered from the liver, spleen and kidney, and infrequently from heart blood as well. These data

suggest that bacterial L-forms may be present and detrimental in immunologically-suppressed individuals.

AI-08724-02 L. W. Wannamaker (University of Minnesota)

This Principal Investigator has reported that streptocin A, the bacteriocin of a group A streptococcus, can also be found in a cell-associated form in addition to its extracellular location, in broth cultures. The specific M protein could be readily separated from the streptocin A. Genetic studies have indicated that determinants for production and for immunity of cells to streptocin A are both located on a single plasmid. Loss of both properties occurred after treatment by chemical curing agents. Additionally, a B group streptococcus also produced streptocin B - this existed in two different molecular forms. Group B streptococci produce three extracellular nucleases of three distinct types; production is independent of the group B streptococcal type. All of these B streptococcal nucleases, however, are immunologically distinct from the group A nucleases, and must be understood in interpreting serological responses following infection with either type of streptococcal organism.

AI-06487-12 D. W. Watson (University of Minnesota)

This productive investigator has concentrated his efforts on study of the group A streptococcal pyrogenic toxin (SPE). These toxins are related to the erythrogenic toxins and aid in induction of erythema. Other toxin manifestations include enhancement of lethal endotoxin shock, blockage of the RES system, suppression of antibody response and mitogenic activity for human and rabbit lymphocytes. Partial purification of the toxin showed a high content of hyaluronic acid as well as several proteins. The most interesting toxin activity, now under intense pharmacologic investigation, is the enhancement of endotoxin activity by the SPE. The SPE, when placed directly on cat cerebral cortex, caused brain edema and alteration of membrane permeability; the SPE could therefore enhance endotoxin by disrupting the blood-brain barrier.

AI-12273-03 G. A. Leslie (University of Oregon)

The overall goal of this project is a study of the

modulation of the immune response to group A streptococci. Specific emphasis is placed on anti-streptococcal A carbohydrate (SACHO) immunity in a rat animal model system; this is being explored to gain a better understanding of the cardiac pathology of streptococcal sequelae. Gel diffusion data indicates that the ID-1 idiotypic antibody determinant is present on the L chains of anti-SACHO antibodies. Seven inbred rat strains were hyperimmunized with the streptococcal vaccine. All seven inbred strains were classified as "low" antibody responders, suggesting that the immune response genes for SACHO may not be closely linked with the major rat histocompatibility locus Ag-B. To investigate the possible primordial nature of the ID-1 gene, anti-SACHO antibodies from rabbits, goats, chickens, quail and sharks were analyzed for the presence of ID-1. The inability to detect it on these antibodies suggests the species specificity of ID-1. These observations provide evidence for a genetic predisposition in the production of either precipitating or nonprecipitating antibodies to SACHO. Of potential importance is the observation that the magnitude of the immune response to SACHO is not necessarily correlated with the major histocompatibility locus of the rat.

AI-12219-02 H. C. Dillon, Jr. (University of Alabama)

This investigator has been studying a number of new streptococcal serotypes and determining their characteristics. He has reported a new type, M-65, distinguished from other virulent M-types. This M-65, found in pyoderma and pharyngitis, was a predominant pyoderma strain in 1974. A second probable new type, Truro-12, was identified both in Alabama and in England. It may be of considerable epidemiologic importance, as it was also recently identified as the cause of an epidemic in a newborn nursery in Texas; it therefore may be widespread epidemiologically. Typing of group A streptococcal strains from pediatric clinics, to monitor current epidemiology, showed that four of the M-types accounted for 61% of throat strains. Data obtained in this study indicate also that M-antigens differ significantly antigenically in relation to site of infection. Pyoderma streptococci may produce less M-antigen (and presumably require less for virulence) than do the usual pharyngeal streptococci. M-antibody, especially serum antibody, may be of less importance in pyoderma than in streptococcal pharyngitis.

AI-08429-10 T. J. Kindt (Rockefeller University)

The antigens of the hemolytic streptococci and the immune response to them are still a subject of considerable medical importance. It is now widely accepted that in some unknown manner streptococcal immunity is definitely involved in the pathogenesis of both acute glomeruler nephritis and rheumatic valvular heart disease. Results of studies on these group A streptococci, summarized here, show that antibodies to the peptidoglycan moiety of the streptococcal cell wall are elevated in patients following a streptococcal infection. They are also more commonly elevated in patients with juvenile rheumatoid arthritis. Further studies are needed to determine if this indication of a bacterial infection is involved in the pathogenesis of juvenile rheumatoid arthritis.

Research projects on a number of other infectious diseases, although not identified as part of any Special Emphasis Program, have been well supported by this Branch. These projects are of significant medical and scientific interest; the research reports indicate that considerable progress continues to be made. Research on cholera, tuberculosis, and leprosy are part of the Institute's overall responsibilities for Disease Control Measures. Some highlights from several of these individual projects are summarized here.

2. Cholera

The significant findings reported previously on the nature and mode of action of the cholera toxin, in causing massive fluid/electrolyte release from the gut wall without significant pathology, has stimulated a number of other research projects on diarrheal disease. Of particular interest are studies on diarrheas caused by enterotoxigenic E. coli.

AI-07631-10 R. G. Freter (University of Michigan)

Adsorption of bacteria or bacterial toxins is regarded as the first step in penetration across the epithelial barrier. The adsorption mechanism is under intensive study by this Principal Investigator, as this appears to be the limiting factor in immunogenicity of oral vaccines. The ability of Vibrio cholerae to adhere to brush borders or intestinal slices was correlated with motility. Non-motile vibrios did not adhere

(as reported previously by others). The adhesion to brush border cells was a reversible phenomenon, as the adherent vibrios could be eluted; this elution was due to loss of adhesiveness, without any apparent effect on mucosal receptors. Additionally, anti-vibrio antibody prevented adhesion. Data also indicate that adhesion of vibrios to brush borders involved at least two mucosal receptors, in addition to simple mechanical entrapment of the bacteria in the mucus.

AI-10885-04 S. Falkow (University of Washington)

The nature of enterotoxin production by E. coli forms the thrust of this research program by a very talented and productive group of investigators. The specific plasmid sequences that encode for the heat-stable (ST) and the heat-labile (LT) enterotoxin of E. coli has been isolated. A number of hybrid clones also were isolated and are being screened to determine if they encode for a polypeptide that can react immunologically with specific anti-LT serum. Such a clone would be an excellent candidate for a vaccine strain. Additionally, progress has been made in genetic analysis of the plasmid encoding for the K88 antigen of E. coli in strains of porcine origin. This plasmid-mediated protein antigen is essential for adherence of host bacterial cells to small bowel epithelium. Expectations are that these strains also can be utilized as potential immunizing agents. The molecular findings from these genetic studies are now on the verge of being exploited for the practical construction of effective vaccine strains.

AI-08877-08 R. A. Finkelstein (University of Texas)

This Principal Investigator has been actively pursuing studies on cholera toxin purification and immunogenicity. He has also been clarifying homologies between the cholera and the E. coli enterotoxins, which are immunologically and actively related. Mutants of V. cholera were isolated that were non-toxigenic; these were fed to volunteers in doses up to $1x10^{10}$ viable vibrios, without causing any detectable untoward effects. The organisms colonized the gastrointestinal tract and could be re-isolated rectally. These orally-immunized volunteers were challenged with virulent cholera vibrios, and were found to be immune to cholera. One drawback is that organisms re-isolated from the volunteers showed evidence of reversion to toxinogenicity. These and

subsequent studies should enlarge our understanding of how this unique protein, the cholera toxin, interacts with eukaryotic cells and activates adenylate cyclase.

3. Tuberculosis

This disease is still a significant cause of death from infectious diseases in the United States. Reports from the Center for Disease Control show that about 30-35,000 cases/year are diagnosed and treated throughout the nation. Despite the considerable amount of funding for research and treatment, tuberculosis continues as a significant public health problem.

AI-01636-19 G. P. Youmans (Northwestern University)

Improvements have been reported in the immunizing activity of mycobacterial RNA preparations. Currently a 0.5 g vaccinating dose gives immunization in mice equivalent to 50 g of the older preparation. In a highly-inbred mouse strain as little as 0.0005 g can be used as a vaccinating dose. Immunogenic activity could be preserved and retained for long periods if the RNA material were lyophilized. Mice vaccinated with the mycobacterial RNA produced a high level of immune response, and did not develop delayed-type hypersensitivity (DTH) to the PPD; additionally, rifampin had no effect on the immune response. Following vaccination of mice with mycobacterial RNA, production of a mycobacterial growth-inhibiting factor (MycoIF) was increased. This MycoIF is a lymphokine from mouse spleen cell cultures that inhibits intracellular multiplication of virulent tuberculosis within normal mouse peritoneal macrophages in vitro. It was also found that a ribosomal fraction from an H37Ra strain of Mycobacteria was an effective inhibitor of tumor growth in mice; this was not due to any direct toxic effect on the mixed tumor cells.

AI-07485-09 D. W. Smith (University of Wisconsin)

A guinea pig model of tuberculosis (TB) was used to study the efficacy of BCG vaccinations against TB. Vaccinated guinea pigs showed good protection for periods as long as six months post-challenge; no hematogenous transport of bacilli back to the lungs of the vaccinated animals was observed. BCG-vaccinated guinea pigs surviving an infection by the respiratory route were superinfected intravenously 28 days afterward. Results of these studies showed that the lungs of vaccinated infected guinea pigs do not possess a bactericidal activity against virulent Mycobacteria.

The animal model developed in this study appears to approximate closely the early events in pathogenesis of TB in man. BCG vaccination significantly reduces and retards transport of virulent bacilli back to the lung; this could explain the mechanism by which BCG protects man against TB.

AI-08190-09 J. K. McClatchy (National Jewish Hospital, Denver)

The goal of this project is a study of the mechanisms of action of the most extensively used anti-tuberculosis (TB) drugs. A significant amount of cross-resistance between three of the most commonly-used anti-TB drugs, capreomycin, viomycin, and kanamycin, was found. It is obvious that the order of drug administration in a re-treatment chemotherapy program for TB should be based on appropriate drug susceptibility tests and drug toxicity considerations. Mutants of the nonpathogenic M. smegmatis have been isolated that are

susceptible to the amino-glycoside antibiotics; studies are now in progress on the cross-resistance problem using this less hazardous strain. Previous study had indicated that Mycobacteria susceptible to pyrazinamide (PZA) incorporate the drug and convert it to pyrazinoic acid (POA). In an acidic environment, the POA is then responsible for the anti-TB activity of PZA. Susceptibility testing in the low pH range is difficult; a thin layer chromatography technique was therefore developed that was satisfactory for susceptibility testing. Several species of rapidly-growing Mycobacteria have been increasingly implicated in human disease, especially in immunosuppressed patients. These organisms (M. abscessus, M. chelonei, and M. fortuitum) are highly resistant to most of the anti-TB drugs now available, as the results of drug testing show.

4. Leprosy

AI-11204-02 W. F. Kirchheimer (U.S.P.H.S. Hospital, Carville, La.)

This investigator has concentrated his efforts on study of the native nine-banded armadillo as a suitable test animal for studies on leprosy. He reported earlier that this animal, because of its peculiar physiology and relatively undeveloped immune system, could serve as an animal model for growth and pathogenesis of the Mycobacterium leprae. Current results indicate that immature armadillos have the same susceptibility to challenge with M. leprae as do the mature animals. Increasing challenge doses correlated directly with increasing percentage of death of test animals and with decrease in survival time of the challenged animals. Under standardized conditions, challenge with $2\text{-}7 \times 10^8$ organisms caused death in 80% of the animals before 848 days, with a mean time of 800 days. A standardized intracutaneous infecting dose that will not result in disseminated infection in 90% of test animals is now under study.

Wild-caught armadillos in southwest Louisiana were shown to be infected in their natural state with acid-fast bacteria resembling M. leprae. This poses a number of questions regarding the usefulness of the armadillo, and whether a public health problem may possibly exist in regard to an animal reservoir of human leprosy in this area. These questions are not

fully resolved as yet, although the Center for Disease Control is of the opinion that human cases of leprosy have no relation to any animal reservoir.

D. Biology and Pathogenesis

A variety of other projects, not specifically directed toward any targeted program, are also supported by the Branch. These grants are wide-ranging and cover a broad array of subject matter, many of them of a very fundamental nature.

Some representative programs are summarized here.

AI-02430-18 J. K. Spitznagel (University of North Carolina).

The broad objective of this program is to study the structure, modes of action and antimicrobial capacities of the enzymes and other constituents of polymorphonuclear leukocytes (PMN). Results have clarified the composition of specific granules of the PMN- each granule class comprises about 16% of the cytoplasmic protein, all of the lactoferrin and about half of the lysozyme of the PMN. The azurophil granule contains the myeloperoxidase (MPO), half of the lysozyme and most of the β-glucuronidase. It was also found that both the specific and azurophil granules of human PMN degranulate in response to phagocytosis. Dr. Spitznagel has shown that hydroxystilbamidine and pentamidine can suppress the immune response. Both of these diamidines have been used to treat fungal and protozoal infections, where the parasites invade reticuloendothelial cells. The immunosuppressive action of the diamidines appears to involve action on macrophages; the diamidines also inhibit proteases and stabilize the lysosomes. These results show the importance of interactions between phagocytic cells and other immune mechanisms in infections.

AI-12158-02 D. Pappagianis (University of California, Davis)

This project concentrates on the role of lymphocytes in immunity to Coccidioides immitis. This microorganism causes a fungal disease of considerable epidemiological significance in the desert and dry areas of the western United States, especially California, Arizona and New

Mexico. The infection can be particularly severe, with a mortality rate of about 10%. Two new assays were developed, based upon serological and cellular response to killed spherules; a radioimmunoassay that measures the binding of mouse γ-globulins to spherules via I^{125}--labeled rabbit antimouse γ-globulins, and a spleen cell mixture with Cr^{51}-labeled mouse RBC with or without spherules, measuring the percent of chromium released after two hours. The data show that, in the first test, the γ-globulins from mice vaccinated with spherules or infected with arthrospores bind to a greater extent than do control animals. In the second test, normal spleen cells plus RBC and spherules release about 6% of the labeled chromium, while immune spleen cells from chronic infections, plus RBC and spherules, release 60% of the chromium. Search continues for a more successful immunogen for vaccinating mice with C. immitis, based on the postulate that cell-mediated immunity is a more effective response against infection and that such a response could be selectively stimulated. Preliminary data show that diketene reacts with killed spherules and that the resulting vaccine was more effective in protecting mice against a lethal challenge of arthrospores. Another method has been the digestion of spherule walls by chitinase as an alternative to killed spherules. This preparation is less bulky and, by removing chitin, it is anticipated that cryptic antigens may be uncovered that can be more readily modified for use as a potential vaccine.

AI-11865-06 C. L. Duncan (University of Wisconsin)

Clostridium perfringens is recognized as a major cause of foodborne disease in the United States, yet little is known of this type of food poisoning. Results of this project show that a specific enterotoxin is responsible for the symptoms. This toxin, purified by affinity chromatography, has a characteristic protein peak and is essentially free of contaminating materials; it has a molecular weight of about 35,000 daltons. Studies on the mode of action of the toxin, in rat ileal sacs, showed that toxin inhibited oxidative metabolism. Highly-purified toxin also showed histopathological activity; unlike cholera, E. coli or Shigella enterotoxins, epithelium was denuded from the tips of ileal villi at concentrations of the enterotoxin necessary to induce fluid accumulation in the rabbit. Again, unlike cholera toxin, perfringens enterotoxin had

no effect on cyclic AMP levels in intestinal epithelium. It is hypothesized, from these data, that the perfringens enterotoxin acts via membrane damage causing subsequent inhibition of cellular function.

III. VIRAL DISEASES BRANCH

The approximate level of support provided to Viral Diseases Branch:

Type of Grant	Number	Amount
Research Grants[1/]	250	$15,706,764
Training	14	1,013,048
Fellowships	41	585,404

Summary

It is well established that viruses are the underlying or direct cause of numerous diseases of man ranging from the common cold to fatal encephalitis. We are rapidly gaining fundamental and essential knowledge of the biology of viral infections and the host's responses to these pathogens. This continually expanding base of knowledge has already provided the technology necessary for the control or irradication of important public problems such as smallpox, rubella, and polio. Yet the mechanisms by which these truly obligate parasites selectively infect tissues and cause disease are not completely understood. For most viral diseases specific diagnosis by isolation of the virus is still difficult, cumbersome, slow, and expensive. Even when diagnosis is made, usually there is little in the way of available therapy to interrupt these infections and hasten recovery. It is these remaining problems that the program areas of the Viral Diseases Branch attempt to address.

Five special emphasis programs are currently included: Influenza, Viral Hepatitis, Chronic and Degenerative Diseases of Man, Clinical Virology and Antiviral Substances.

A. Influenza (SEP)

Influenza has loomed again this year as a serious threat and public health problem. The A/Victoria strain of influenza virus touched off illness in every county in the nation and reached epidemic proportions in several states. In addition, a new variant of the virus, antigenically similar to the swine strain was found to be circulating within a military population at Ft. Dix, New Jersey. The potential consequences of this swine-like variant spreading into the U.S. population,

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1/ Includes 24 Career Awards ($586,742)

which is almost totally susceptible, spurred the inauguration of a federal program for producing, testing, and distributing vaccine for "swine flu" and the monumental and unprecedented task of immunizing 200 million people. The technology and fundamental information needed to achieve these objectives has become available largely through programmed research on influenza viruses in this country as well as in others. During the last year, research supported through grants from NIAID has contributed significantly toward the advancement of our knowledge of the viruses that cause influenza and means of controlling the disease.

AI-09304-08 E. H. Kilbourne (Mt. Sinai School of Medicine) and AI-11823-02 P. M. Palese (Mt. Sinai School of Medicine)

Drs. Kilbourne and Palese have been studying the ribonuleic acids (RNA) of influenza viruses to elucidate the mechanisms of replication leading to genetic recombination and antigenic drift in these viruses. A better understanding of these mechanisms is important in the development of more effective and safer influenza vaccines.

Analysis of the segmented RNA's of influenza A viruses by electrophoresis on polyacrylamide urea slab gels has provided a method for sharper resolution of the number and migration rates of different segments than previously has been possible. Using this system, the RNA genome of influenza virus can be resolved into 8 bands. Comparison of the RNA patterns produced by two different antigenic strains of influenza viruses also reveals major differences in migration speeds of different bands and analysis of the RNA's of a recombinant virus derived from these two strains permits the identification of RNA segments which have been derived from one particular parent. By extension of these techniques it has been possible to define which RNA segment codes for specific viral protein and to analyze recombinant strains to identify which genes have been derived from each of its parents. The hemagglutinins and neurominidases are two proteins that have been identified as being coded for by specific RNA segments.

AI-10256-05 M. W. Pons (N.Y. Public Health Research Institute)

Several observations similar to those of Kilbourne and Palese have been reported by Dr. Pons who has also shown on slab gel electrophoresis that the viral genome consists of 8 distinct segments of single-stranded RNA in clusters of

three, three and two bands. Similarly, viral specific double-stranded RNA found within infected cells also consists of eight segments with the same clustering. These double-stranded RNA segments apparently replicate simultaneously rather than sequentially. Thus replication of one segment does not depend on the prior replication of another strain. Pons has also shown that the viral messenger RNA associated with polysomes in the infected cells is not free RNA but is bound to protein (RNP).

AI-11614-02 O. M. Rochovansky (N.Y. Public Health Research Institute)

Dr. Rochovansky has extended the observations of Pons to demonstrate that the viral ribonucleoprotein-polymerase (RNP+P) complexes in infected cells can be separated chromatographically into three size classes. These purified complexes have been shown to be infective when all three size classes are mixed but are noninfectious individually. The classes can be interchanged with similar classes from other variants and still retain infectivity when all three classes are present. Even though most of the observations are preliminary results, these developments can be projected into approaches for "tailor-making" influenza viruses from known strains, variants and mutants into recombinants that have characteristics most desired for vaccines. Certainly the direction has been indicated for such investigations in the immediate future.

AI-08831-08 R. G. Webster (St. Jude Children's Research Hospital)

Dr. Webster has demonstrated that recombination may occur among animals or birds in nature. Turkeys were mixedly infected with turkey influenza virus (TIV) and fowl plague virus (FPV). Recombinant influenza viruses possessing the hemagglutinin of FPV and the neuraminidase of TIV became the dominant virus present in those birds and killed them. These recombinant influenza viruses spread under natural conditions of transmission to contact turkeys and caused a miniepidemic of disease. These studies suggest a natural mechanism of recombination, selection, and transmission consistent with the emergence of new pandemic strains of human influenza viruses.

B. Viral Hepatitis) (SEP)

A vaccine for hepatitis B, a continuing public health problem,

is undergoing testing for safety and efficacy. This killed, subvirion particle vaccine for active immunization and specific immune globulin for passive immunization are certain to reduce the incidence of hepatitis B in this country among high risk groups. It is significant that this vaccine is being developed without an acceptable means of propagating the virus in vitro and with no experimental host other than the chimpanzee. The course of the viral antigen used in this vaccine is blood of human chronic carriers. Similar and possibly more formidable obstacles face the development of a vaccine for hepatitis A. The antigen for hepatitis A virus has been found only in human stools and the livers of experimentally infected marmosets. A South American marmoset and the chimpanzee are the only species, other than man, uniformly susceptible to this virus and the availability of both are extremely limited. These caveats in technology severly hinder progress in the investigations of these viruses and the diseases they inflict on man. Fortunately, serological markers are now available for both viruses that can be used in diagnosis and epidemiological studies.

AI-11860-06 D. H. Carver (Johns Hopkins University)

Dr. Carver has been attempting to improve upon the cell culture assay that he developed for hepatitis B infectivity. This assay involves the ability of hepatitis B virus to inhibit replication of another virus used as a bench mark by means of a phenomenon called intrinsic interference. The use of growing cells for the production of intrinsic interference by hepatitis B virus was assessed using infectious Dane particles prepared by an NIAID contractor as the source of hepatitis B virus. Preliminary data indicated the cells inoculated with virus at a point when the sheet is 50% confluent may develop intrinsic interference at a more rapid rate and to a greater extent than cells inoculated when fully confluent. Also, the pretreatment of cells by pulsing with 40% calf serum for as little as 6 hours before viral inoculation and for 14 hours after inoculation appeared to enhance optimally the production of intrinsic interference by the Dane particles.

The MS1 prototype virus of hepatitis A did not appear to induce intrinsic interference in a monkey kidney line (MA134). Thus, there still is no in vitro assay for infectivity of hepatitis A virus.

C. Chronic and Degenerative Diseases of Man (SEP)

Research on the chronic and degenerative diseases have centered on recovering the causal agents, determining the time of onset of the disease and defining host factors which influence the pathological evidence of disease.

AI-01475-19 E. H. Lennette (California Department of Health)

Drs. Lennette and Cremer have been examining tissues from patients with amyotrophic lateral sclerosis. No virus-like structures were observed in the pons, dorsal medulla, and ventral part of the cervical spinal cord, but crystalline arrays of virus-like particles were detected in the muscle tissues of one patient. However, no virus was isolated by these investigators from any of these tissues even after extensive co-cultivation and passage in cell cultures.

In a previous report Lennette and Cremer described three patients who were congenitally infected with rubella virus and developed atypical demyelinating disease in their second or third decade of life. Attempts to isolate virus were recently successful from one patient, a 12-year old child. The virus was identified as rubella virus. The isolation of this virus demonstrated that rubella virus can be added to the growing list of viruses causing "slow infections" of the central nervous system.

AI-12203-02 F. Rapp (Pennsylvania State University)

Measles virus is involved in the pathogenesis of acute encephalitis, subacute sclerosing panencephalitis, and possibly multiple sclerosis. It has been suggested by other investigators that a mutant or defective variant of measles virus may be the etiologic agent of SSPE. Dr. Rapp and co-workers determined the encephalitogenic potential of nine temperature sensitive (ts) mutants of measles virus in newborn hamsters. The parental virus produced acute encephalitis without prior adaptation. Six of the mutants were attenuated, two were virulent, and one was associated with hydrocephalus with acute onset. The hydrocephalus-inducing mutant was genetically distinct from the others. The development of a moderate degree of hydrocephalus among a minority of animals inoculated with certain mutants is suggestive of a slow developmental process. It was concluded from the data that the mechanisms of attenuation most probably do not involve a temperature induced inhibition

of virus replication, but rather appear to be related to the partial defectiveness of the mutants under permissive conditions.

AI-09484-07 M. B. Oldstone (Scripps Clinic and Research Foundation)

In other studies on SSPE, Dr. Oldstone and co-workers have been investigating the involvement of the immune system in the disease. According to a recent report, a number of human cell lines, including epithelial, neural, glial, and lymphoid cells, infected with several strains of measles virus were found to be disrupted upon incubation with fresh sera from humans containing antibody to measles virus. In all instances the disruption was mediated by the alternative complement pathway, rather than the conventional pathway, but they concluded that the slow degenerative disease that is characteristic of SSPE does not appear to be associated with an immunologic defect in this complement mediated system. It is noteworthy that high titers of measles antibody exist in SSPE patients while measles virus also persists in these individuals. This paradox suggests that SSPE patients do have a specific immunologic defect even though none has been found yet. Oldstone and his associates have found evidence in vitro that immune injury to infected cells may be suppressed by a phenomenon called antigenic modulation. Upon the addition of antibody to mealses virus, viral antigens expressed on the surface of infecied cells can be modulated from the cell's membrane. Removal of measles antigens from the surface occurs rapidly and is accompanied by a parallel reduction in the lysis of these cells by antibody and complement. Protracted exposure of acutely infected cells to measles virus antibodies results in a population of cells that appear and grow normally. These cells continue to express viral antigens internally, but not at the cell surface and are refactory to immune lysis. These observations may explain the persistence of virus in spite of a vigorous, host antiviral immune response in certain chronic infections of man.

AI-06246-12 J. G. Stevens (University of California, L.A.)

Despite the evidence in man for reactivation of latent herpes viruses, attempts to demonstrate this reactivation in experimental animals has until recently failed. Dr. Stevens and his associates instilled Diplococcus pneumonia intratracheally into mice harboring latent herpes

simplex infections in sacrosciatic spinal ganglia. All mice developed a severe pneumonia and were given penicillin therapy. Survivors were serially sacrificed and tissues examined. Herpes simplex virus was reactivated in the ganglia and appeared to travel proximally and distally in associated nerve trunks. However, clinically apparent disease due to herpesvirus was not detected in any mice.

D. Clinical Virology (SEP)

AI-11611-03 D. M. Horstmann (Yale University)
AI-08731-06 J. C. Niederman (Yale University)

Infectious mononucleosis (IM) is a disease of adolescents and young adults caused by a herpesvirus. Drs. Horstmann and Niederman have been studying the epidemiology of the disease with particular emphasis on its transmission and detection in susceptible populations. Throat washings from IM patients in a closed population at West Point revealed that over half contained the putative agent (EBV) up to six months after illness. In one case shedding of virus was detected 16 months after onset of disease. Not all patients shed virus and some excrete virus only intermittently for three to four months. This carrier state after clinical illness and presumably after inapparent infection helps to explain the difficulties encountered in tracing transmission on a case-to-case basis. These observations also indicate that there is a large pool of carriers of EBV in the population capable of transmitting the virus through appropriate exposure. The site of virus shedding has been determined to be the mucosa of the mouth and the salivary glands and the saliva is probably the vehicle for dissemination of the virus in nature. For detection and diagnosis of IM, three new antibody tests were compared with the standard heterophile antibody test for sensitivity of detection, specificity of the reaction and for persistence of antibody in confirmed IM cases. From the results the investigators concluded that the standard test utilizing sheep red blood cells should be replaced with the newer heterophile antibody test employing beef hemolysin or agglutination of horse RBC. Another new test that is specific for IgM antibody to EBV and highly sensitive was considered too difficult to perform to be of routine diagnostic value. The latter is based on the ability of serum to neutralize the transforming potential of EBV for fetal human lymphocytes.

AI-01475-19 E. H. Lennette (California Department of Health)

A variety of techniques have been described for type-specific identification of herpes simplex virus (HSV) antibodies in human sera, but most have had significant limitations as being either too nonspecific or too cumbersome or costly for routine use. Drs. Forghani, Schmidt and Lennette have reported the development of an indirect solid phase radioimmunoassay for typing of antibodies to HSV types 1 and 2 in human sera. The test is based on the absorption of sera with uninfected, HSV-1-infected, and HSV-2 infected cells and testing for residual antibody. The high sensitivity of the test permits examination of sera at high dilutions, and thus relatively small volumes of virus-infected cells are required for cross-absorption of antibodies. The test was highly suitable for detecting HSV antibodies in cerebrospinal fluid and for detecting two types of HSV antibody in the same serum specimen. It was possible to show that a marked, type specific antibody response to HSV-2 does occur in individuals with a primary HSV-2 infection who have experienced a prior infection with HSV-1.

AI-09785-06 A. H. Ross (Nassau County Medical Center)

Zoster immune globulin (ZIG) has been re-evaluated in high-risk, debilitated, and age-dependent patients as a prophylactic treatment against herpes zoster, a very painful and sometimes fatal disease in adults. The ZIG reduced the severity of disease as measured by the number of pox developing in patients but did not alter the incubation period or the contagion of infected patients. It was concluded that ZIG may be insufficient to curb an outbreak in a hospital population, especially among patients whose immune system has been compromised. ZIG given 4 to 9 days after exposure was as effective as within the first 3 days. The investigators have revised their recommendations for current ZIG usage. For prevention in high-risk, immunosuppressed patients 0.15 ml/kg ZIG is recommended. For attenuation of symptoms, 0.05 ml/kg ZIG is suggested. They conclude that with prescreening by the fluorescent-antibody-membrane-antigen technique it should be possible to treat preventively all adolescents and adults known to be at risk with known exposures. Until a vaccine or a highly effective chemotherapeutic agent supplements or replaces ZIG, this will probably remain the first line of defense against varicella or herpes zoster.

E. Antiviral Substances (SEP)

Research support on antiviral substances is primarily concerned with interferon, its induction and its mechanism of action. Support is also provided for identification and characterization of other effective antiviral agents.

AI-05629-14 T. C. Merigan (Stanford University)

Work continues to relate interferon to the immune response, examining both the production of interferon on an immune specific basis by sensitized lymphocytes responding to specific antigen and the influence of interferon on the immune responses in vivo and in vitro. Such studies relate directly to their clinical trials of human interferon in viral and malignant diseases.

The interferon produced by human T-lymphocytes has different physical chemical characteristics than the interferon produced by diploid fibroblasts, leukocytes, or even purified blood mononuclear cells stimulated with herpes simplex antigen. Studies have indicated that the interferon produced in herpes zoster vesicles is more like the luekocyte interferon than the so-called "immune" interferon produced by antigen sensitized T-cells. A study has been completed comparing interferon and lymphocyte transformation in purified T-lymphocyte and macrophage cultures and in purified mononuclear cell preparations. Although greater interferon responses were noted after recent herpes simplex lesions with both kinds of cell responses, only the T-lymphocyte response was shown to be wholly immune specific in nature, that is, dependent on prior infection of the host. Work has been completed with Drs. Tak Wiktor and Hilary Koprowski of the Wistar Institute to examine the role of interferon production in the efficacy of Wistar vaccine in protecting primates from rabies challenge. Interferon production was most marked with the Wistar preparation as compared to several other types of vaccine. However, the use of interferon inducers to augment interferon production by other vaccines failed to equal the protective effect of the Wistar vaccine.

AI-12170-02 S. B. Greer (University of Miami)

Two halogenated pyrimidine nucleosides, 5-bromodeoxyuridine (BUDR) and 5-iododeoxyuridine (IUDR) are effective inhibitors of viruses that contain deoxyribonucleic acids (DNA) such as herpes simplex virus. The toxicity of these drugs limit

their effectiveness. Dr. Greer has been comparing halogenated analogues of deoxycytidine with the deoxyuridine analogues for antiviral effects against herpes simplex virus in cell cultures.

5-bromodeoxycytidine and 5-iododeoxycytidine inhibit the replication of herpes simplex virus as effectively as BUDR and IUDR. However, the 5-halogenated analogues of deoxycytidine are 1-100 times less toxic to uninfected cells than similar analogues of deoxyuridine. The selective action of the halogenated analogues of deoxycytidine appears to be the result of a virus-induced enzyme that converts the halogenated analogues of deoxycytidine to halogenated analogues of deoxycytidylate. The results of these studies indicate that the 5-halogenated analogues of deoxycytidine are more selective inhibitors of herpes simplex virus replication than the 5-halogenated analogues of deoxyuridine.

AI-01992-17 M. N. Oxman (University of California, San Diego)

One new treatment method, based on in vitro investigations, animal studies, and some circumstantial evidence, is photodynamic inactivation of herpesvirus. This is achieved by treating the herpes lesions with a dye, such a neutral red, and then exposing the lesions to light. However, it has been shown that photodynamically inactivated HSV can induce cancerous changes in cells in vitro, suggesting that this form of treatment is not without hazard.

The results of recent studies revealed that the neutral dye and the placebo-treated patients did not differ significantly in the duration of their discomfort or in the time to crusting or complete healing of their lesions. Furthermore, treatment with the neutral red dye did not demonstrably affect the frequency of or length of interval between subsequent recurrences of herpes infections.

Since this placebo-controlled clinical study failed to demonstrate the efficacy of neutral red dye and light in the treatment of recurrent HSV infections and since a potential carcinogenic risk is involved, the scientists recommend that routine use of this form of therapy be discontinued.

AI-09785-06 A. H. Ross (Nassau County Medical Center)

Dr. Ross has investigated the efficacy of adenine arabinoside (vidarabine) as an antiviral drug in the treatment of 36 patients with varicella-zoster infections and 8 with herpes simplex virus infections at doses ranging from 10 to 20 mg/kg/day for periods of 5 to 10 days. Their results have been interpreted subjectively as indicating a positive value of active vidarabine treatment in immunosuppressed patients with varicella pneumonia and in patients with disseminated herpes simplex virus infections other than herpesvirus encephalitis. However, six forms of moderate reversible toxicity due to vidarabine were noted thereby challenging the reputation of this drug as being non-toxic.

F. Biology and Pathogenesis

It is essential that there be a continuous gathering of new knowledge on the biology of viruses so as to better elucidate the mechanisms by which they infect man or other hosts and produce disease. Different approaches in investigations of viruses often result in new insights into their detection, pathogenesis, and control of the diseases they induce.

AI-09706-05 H. Koprowski (Wistar Institute)

Rabies virus replication in suckling and adult mouse brains inoculated with either fixed or street virus was studied by light and electron microscopy. Chronologic study of fixed virus-infected suckling mice showed that virus budded from the plasma membrane of neuronal cell processes and around the nuclei prior to the development of virus particles in the cytoplasma of cells. Nucleocapsids and virions were observed exclusively in nuclear areas and cellular processes of neuronal cells and, on rare exception in an astrocyte indicating high neurotropism of rabies virus regardless of the strain of virus and the age of host animal. Virus transmit between contiguous neuronal cell processes was observed in suckling and adult mouse brain after both street virus and fixed virus infection. Nucleocapsids and virions were also found in myelinated axons in addition to the unmyelinated neuronal cell processes.

The implication of cell to cell spread of virus within the central nervous system in rabies virus infection means that the pathogen can spread without the interference of viral antibody or phagocytes.

AI-10217-06 L. A. Glasgow (University of Utah)

Nonspecific immunotherapy with Corynebacterium parvum (C. parvum) or BCG used for treatment of human malignancies, has multiple effects on humoral and cell-mediated immunity as well as the reticuloendothelial system. Viruses, particularly herpesvirus, are an increasingly frequent cause of complicating infections in this patient population. Experimental models have been utilized to define the effect of nonspecific immunoenhancement on viral infections of mice. C. acnes was effective in protecting mice against all four viruses tested (herpes simplex, cytomegalovirus, encephalomyocarditis virus and Semliki virus) and was more effective than C. parvum or BCG against the herpesvirus infections. These data indicate that nonspecific immunotherapy is associated with enhanced resistance to viral infections in mice, and suggest the possibility of similar effects in man.

AI-05600-13 P. W. Choppin (Rockefeller University)

Actin and myosin, the major structural elements of muscle, have been identified as constituents of a wide variety of non-muscle eukaryotic cells. Evidence from several laboratories indicates that actin filaments may provide a cytoskeleton in eukaryotic cells. A protein in preparation of highly purified virions of oncornaviruses and paramyxoviruses has been identified that comigrates with skeletal muscle actin (M.W. 43,000 daltons) in SDS-polyacrylamide gel electrophoresis (PAGE). This protein is present in several oncornaviruses including the transforming Rous sarcoma virus (Schmidt-Ruppin strain), a non-transforming derivative of the same strain, avian leukosis viruses (RAV-1, RAV-2, RAV-7), Rauscher leukemia virus, woolly monkey virus, and gibbon ape virus. It has also been found in Sendai, SV5 and Newcastle disease viruses. It appears that actin is a host protein that is incorporated into viral particles during their maturation. Its function in virus maturation or replication has not yet been determined.

AI-01475-19 E. H. Lennette (California Department of Health)

An animal model for varicella in man has been proposed in collaborative studies between the California State Health Department and Tulane University. A herpesvirus (Delta herpesvirus) produces a disease in patas and macaque monkeys that mimics varicella in man. A close antigenic

relationship has been demonstrated with varicella zoster virus by reciprocal cross-neutralization of the two viruses in convalescent sera from both patas monkeys and man. If it can be shown that the Delta herpesvirus becomes latent in the patas monkey and can be reactivated it might serve also as a model for zoster. More immediately, this model has the potential for investigating immuno-and chemotherapy in acute infections with these viruses and for development of vaccines. Varicella-zoster has the greatest morbidity of all the human herpesviruses. It is anticipated that research on varicella and zoster can now progress at a more rapid pace through the application of this model.

IV. PARASITOLOGY AND MEDICAL ENTOMOLOGY BRANCH

The approximate level of support given the branch:

Type of Grant	Number	Amount
Research Grants[1/]	161	$7,218,553
Training Grants	7	493,215
Fellowships	20	312,967

Summary

Since the last report was prepared (FY 1975) the program has increased slightly in number of grants and funding.

In October, 1975, the workshop recommended by the peer evaluation of the Biological Regulation of Vectors program took place in Easton, Maryland. Currently, a workshop recommended by the evaluators of the program on Immunology of Parasitic Infections is in the planning stage.

In July, 1975, Dr. Delappe gave a talk on the Biological Regulation of Vectors program during an interagency conference on federal research: Health Effects of Pesticides. The conference took place at Crystal Mall, Arlington, Virginia, and the proceedings will be published shortly.

A. Methods for Other Diseases

1. Biological Regulation of Vectors (BRV) (SEP)

For the past 30 years, control of pests and disease vectors has been based primarily on the use of synthetic organic compounds which had the "advantages" of long residual action and toxicity to a broad spectrum of target organisms. It has now been shown that because of these very characteristics many of these pesticides are more deleterious than beneficial, when all effects on man and his environment are considered. Furthermore, resistance to broad spectrum chemical pesticides has reduced their effectiveness in many vector control programs. For these reasons, the search for alternative methods of pest control has become imperative, and it is generally agreed that the best

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1/ Includes 9 Career Awards ($223,236)

approaches will consist of integrated pest management programs which combine biological control, in the broadest sense, with judicious use of more specific chemicals and management of the physical environment. This type of approach to vector control must be based on adequate information about the ecology of the target organism, the environment in which the control program is to be conducted, effects of control measures on non-target organisms in the environment, and the biology of the disease organisms being transmitted.

This program has as its goal the advancement of fundamental studies which might lead to effective methods of biological regulation of vectors.

An evaluation of our BRV program took place in June, 1974. At that time the evaluators recommended that the Institute sponsor a workshop on topics in this area. In October, 1975, the workshop, chaired by Dr. John Briggs of Ohio State University, was held in the Tidewater Inn at Easton, Maryland. Invited participants discussed:

(1) Isolation and development of bacterial pathogens of vectors,

(2) Bacterial diseases of vectors,

(3) Mode of action of Bacillus thuringiensis,

(4) Isolation and development of fungus pathogens of vectors,

(5) Significance of the pathogenicity for animals and plants,

(6) Biochemical basis of invertebrate host susceptibility to fungus infections.

Proceedings of the workshop are now being prepared in final form for publication by the U. S. Government Printing Office and should be available by late 1976. In addition to the presentations by participants, the verbatim text of discussions with 12 invited observers will be included in the proceedings of the workshop.

Specific examples of research progress during the current fiscal year follow:

AI-12894-01 J. B. Kitzmiller (Florida Medical Entomology Laboratory)

For many years this group headed by Dr. Kitzmiller has been in the forefront of work on the genetics, cytology and ecology of natural mosquito populations. Perhaps the most significant recent achievement of this research is their ability to distinguish malaria vector from nonvector populations in Venezuela and Colombia by means of salivary gland chromosome inversions. The vector populations in Venezuela and Colombia differ by an X-chromosome inversion from all other (nonvector) populations. This research could have important implications for the control of malaria in these areas.

AI-06587-12 M. W. Provost (Florida Medical Entomology Laboratory)

These workers have demonstrated by field studies that certain Culex mosquitoes will not take blood, even under conditions of starvation, if they have not first had a carbohydrate (usually nectar) meal. This proves that sugar feeding can be an important factor in the vectorial potential of disease-carrying mosquito populations.

AI-03235-16 J. N. Couch (University of North Carolina)

The important discovery made by another grantee last year concerning the necessity of a copepod (Cyclops) intermediate host for the life cycle of an infective fungal parasite (Coelomomyces) of mosquitoes has been confirmed in this laboratory. Work is progressing on the world monograph of the parasite, Coelomomyces, and its completion is expected by January, 1977.

AI-00513-22 E. Chernin (Harvard University)

This research indicates that Lymnaea emarginata, a snail which serves as an intermediate host for nonhuman schistosomes is a predator which controls laboratory populations of Biomphalaria glabrata, another snail which acts as the intermediate host for certain human schistosomes. Control appears to be exercised as the result of egg destruction and was not associated with

a reduction of fecundity imposed by crowding. Although previous exposure to B. glabrata egg masses appears to enhance L. emarginata's ability to destroy eggs, "conditioning," in the sense that repeated exposures can be directly correlated with an increase in effectiveness, has not been demonstrated. In this era of environmental sensitivity, alternatives to molluscicides such as this warrant further attention.

2. Immunology of Parasitic Infections (IPI) (SEP)

The complexity of structure and function of parasites has made the study of immunology of these infectious agents exceptionally challenging and rewarding. Exciting opportunities for the elucidation of mechanisms and manifestations of immunological responses to parasites now exist as the result of the impressive developments in immunology in recent years.

Major ultimate goals of studies on immunology of parasitic infections are the development of effective vaccines for the prevention of parasitic diseases (such as malaria, schistosomiasis and filariasis), the intervention in the host response to prevent or ameliorate disease processes which are immunologically mediated, and the development or improvement of immunodiagnostic procedures for parasitic infections, especially as they relate to the immune status of the host.

A related goal of these studies is to contribute to an understanding and solution of basic and clinical problems associated with other disease entities, especially immunological disorders and hypersensitivity states. A number of parasitic infections are excellent models for such studies as: (a) the mechanisms of intracellular immunity; (b) the enhancement or suppression of concurrent infections or tumor development; (c) immunopathological mechanisms; (d) development of disease processes in immunosuppressed or immunostimulated hosts; (e) the biochemical and genetic mechanisms for the development of pathogen variants with different immunological characteristics; (f) the genetic basis for variations in host response; and (g) the role of IgE and other cytotropic antibodies in hypersensitivity.

An evaluation of our Immunology of Parasitic Infections program took place in October, 1974, and as a result of this, the evaluators recommended that the Institute sponsor a workshop on topics in this area. A tentative program has

been formulated and the workshop is scheduled for June, 1977. The program will include presentations and discussions involving immunity in helminth infections, immunopathology in schistosomiasis, eosinophils, cell interactions relating to protozoa and malaria. The proceedings of the workshop will be published.

Specific examples of research progress during the current fiscal year follow:

AI-04717-15 J. S. Remington (Palo Alto Medical Research Foundation)

IgG Antibody Suppression of the IgM Antibody Response to Toxoplasma gondii in Newborn Rabbits. Studies were performed in an experimental rabbit model in an attempt to explore the possibility that the absence of an IgM antibody response in human infants congenitally infected with Toxoplasma may be caused by a suppressive effect of IgG antibody passively transferred from the mother. The IgM antibody response to Toxoplasma gondii infection in newborn rabbits previously injected with IgG Toxoplasma antibody was significantly suppressed as determined by an immunofluorescent antibody assay (IgM-IFA). In newborn rabbits infected with Toxoplasma, IgM-IFA antibody was first demonstrated 15 days after infection and reached a peak titer of 1:128 in two animals on days 20 and 25. In newborn rabbits injected with IgG Toxoplasma antibody and then infected with Toxoplasma, the IgM-IFA antibody response was considerably lower; the highest titer was 1:16 and occurred 25 and 30 days after infection. The results of these studies lend support to the concept that maternally transmitted IgG may suppress the IgM antibody response in the fetus and newborn infant.

Resistance to Mycobacterium leprae in Mice Infected with Toxoplasma gondii. Mice chronically infected with Toxoplasma gondii were resistant to footpad challenge with Mycobacterium leprae. Resistance was manifested by lower numbers of recoverable M. leprae in the footpads of protozoal-infected mice and was enhanced in Toxoplasma-infected mice by a booster injection of Toxoplasma antigen in the infected footpad. The results suggest a major role for the activated macrophage in the control of M. leprae infection.

AI-10842-04 J. F. Williams (Michigan State University)

Weanling rats born of mothers infected with Taenia taeniaeformis were found to be passively protected against homologous challenge. Cross-fostering of normal suckling rats onto immune mothers established that passive transfer occurred via the colostrum and milk. Immunoglobulin fractions containing γ A from immune colostrum were fed to 12-14-day-old rats for 4 days via stomach tube. Significant passive protection against challenge with T. taeniaeformis was achieved with γ A from one of three colostrum pools. The effect of colostral γ A preparations on the infectivity of freshly hatched oncospheres of T. taeniaeformis was measured by the intra-intestinal inoculation of immunoglobulin solutions into isolated gut loops containing hatched eggs of the parasite. γ A from one of three pools of immune colostrum caused a significant reduction in the number of parasites which reached the liver. This appears to be the first time that protective activity against a helminth infection has been achieved with γ A.

A fraction of immune colostrum containing both 7S γ 1 and 7S γ 2 immunoglobulins was found to confer passive protection when inoculated parenterally. In view of the prolonged period of absorption (ca 18 days) of 7S immunoglobulins from the gut by the suckling rat, it seems likely that these antibodies are primarily responsible for the natural passive transfer of protection from mother to young.

AI-10645-04 M. Aikawa (Case Western Reserve University)

The effect of immune serum on the merozoite of Plasmodium knowlesi was studied by electron microscopy. The surface of intraerythrocytic parasites was similar when incubated in culture medium that contain either normal or immune serum. Merozoites that were still within infected erythrocytes before rupture or that had not escaped from the erythrocyte ghosts after rupture were coated with only a small amount of fibrillar material. When extracellular merozoites were incubated in medium with normal serum, a uniform coat covered the entire surface with the free merozoites, including the anterior end. The coat was electron dense, compact and fibrillar and extended approximately 20 nm from the plasma membrane of merozoites. The surface coat on free merozoites

incubated in immune serum was thickened (60 to 100 nm), loosely packed and partially detached from the plasma membrane of the parasite. Occasionally a surface coat was also observed on immature schizonts free of the erythrocyte membrane. Agglutination of merozoites was caused by adherence between the surface coats of adjacent parasites. Agglutination between merozoites occurred at any point on the merozoite surface and was not specifically oriented to the anterior end. In the rare micrograph, where a merozoite was entering an erythrocyte, the surface coat appeared to be pushed off the plasma membrane at the point of entry. The surface coat was not visible on intracellular parasites that had recently invaded an erythrocyte. Repeated washing of free merozoites with PBS, Tris total buffer, or calcium-magnesium-free Tris total buffer plus EDTA did not remove the coat.

The effect of immune sera on the coat indicates that the coat is parasite origin. Although the detailed structure and function of the merozoite surface coat is unknown, it may contain important determinants for the induction of functional immunity.

AI-12146-03 T. Jones (Cornell Medical School)

Studies have been made on humoral and cellular immune responses in mice immunized with an attenuated strain of Toxoplasma gondii. Heat-inactivated antitoxoplasma serum did not cause morphologic changes in the organisms, but did markedly influence their interactions with host cells. Toxoplasmas exposed to antibody were no longer capable of entering fibroblasts or HeLa cells. They were readily engulfed by macrophages, but the antibody treatment strikingly altered the intracellular fate of the parasites leading to killing and digestion of the toxoplasmas in phagolysosomes. Addition of antitoxoplasma antibody immediately after infection of macrophages in vitro had no effect on intracellular multiplication of the organism.

The division time of virulent toxoplasmas in mouse peritoneal macrophages in vitro was markedly prolonged in cells from immunized mice. During the first 2-3 months after immunization, the macrophages harvested from the peritoneal cavity demonstrated this cellular immunity directly; thereafter exposure of the macrophages to immune lymphocytes and toxoplasma antigen, or to supernates from such an interaction was required for

induction of the maximal capacity to inhibit growth of toxoplasmas. Induction of the alteration in macrophages by the lymphocyte product was detectable in 6 hr. and maximal at 18-24 hr.

Cultivation in vitro of macrophages from immunized animals for periods longer than 48 hr. rendered the cells nonresponsive to the immune lymphocyte-toxoplasma product. Macrophages from the peritoneal cavities of normal, nonimmunized mice were also incapable of developing the capacity to inhibit growth of toxoplasmas in response to this product. The nonresponsiveness of normal macrophages, or of macrophages cultered for several days in vitro was not changed by exposure of the cells to anti-toxoplasma serum.

AI-11916-02 W. Trager (Rockefeller University)

This worker investigated the effects of antibodies on the cell surface antigens of amastigote and promastigote protozoa by studying the influence of specific antibodies on the mobility of Leishmania cell surface antigens.

Direct and indirect fluorescent antibody methods were used to detect movement of cell surface antigens in living L. donovani promastigotes and amastigotes. Antibodies developed in rabbits against promastigotes (anti-P) and amastigotes (anti-A) were used in conjunction with fluorescein labelled goat anti-rabbit IgG for indirect staining of both promastigotes and amastigotes. Similarly, sera obtained from L. donovani infected hamsters and fluorescent labelled rabbit anti-hamster IgG were used to stain the parasites indirectly. Fluorescein conjugated rabbit IgG fractions of anti-P and anti-A were used for staining both parasite developmental stages directly. Initially, both directly and indirectly stained promastigotes and amastigotes appeared uniformly fluorescent. Progressive patch formation of surface fluorescence occurred with time, caused by the coalescence of membrane antigens by the cross-linking action of the fluorescein labelled antibodies. Movement of labelled surface antigens culminated with the formation of a fluorescent cap at the cell anterior pole. Promastigotes but not amastigotes showed some bipolar cap formation. Capping was antibody concentration, time, and temperature dependent, and was inhibited with the metabolic inhibitors sodium azide and iodoacetamide. Surface antigens of both parasite stages reappeared in the pellicular membrane within 3

hrs - post capping. These results demonstrate the mobility and fluidity of L. donovani surface antigens. Further, the results show that hamsters infected with L. donovani produce specific antibodies (i.e. IgG) directed against some parasite membrane antigens. The results also suggest a mechanism by which the parasite not only survives in the face of a mounted host immune response but actually utilizes the host specific antibodies to mediate the modulation of its own exposed cell surface antigens.

AI-08163-09 K. Warren (Case Western Reserve University)

The production of a monospecific rabbit anti-mouse eosinophil serum in this laboratory has led to studies of eosinophil kinetics in schistosomiasis, of the role of the eosinosphil in immunity to S. mansoni infection, and of eosinopoiesis.

The kinetics of eosinophilia was studied in mice with different intensities of S. mansoni infection. Since eosinophilia was related largely to egg production by the worms, the effect of isolated schistosome eggs injected into mice by various routes was examined. While the host response was dependent on the route of egg administration, primary peripheral and bone marrow responses were seen on intravenous injection and secondary responses occurred on intravenous and subcutaneous injection. In unsensitized and egg-sensitized mice, eosinophils were first seen around eggs injected into the pulmonary microvasculature at 96 and 24 hours, respectively. When the granulomas were maximal in size eosinophils made up at least 50 per cent of the lesions. Administration of anti-eosinophil serum profoundly suppressed eosinophils in the peripheral blood, eliminated mature eosinophils and markedly increased eosinophil precursors in the bone marrow, and ablated eosinophils from the tissue lesions, considerably reducing their areas.

Partial immunity to schistosomiasis mansoni has been demonstrated in mice, and has recently been transferred passively with serum, but not with cells. In our studies the effect of anti-leukocyte sera in mice partially immune to schistosomiasis was tested in vivo using quantitative assay systems for schistosomula in the lungs and adult worms in the portal venous system. Mice infected with 10 cercariae of S. mansoni 16 and 32 weeks prior to challenge with 500 cercariae showed

reductions in the recovery of schistosomula at 4 and 6 days of approximately 40 per cent; adult worm recovery following exposure to 100 challenge cercariae was reduced by 60 per cent. Treatment with antilymphocyte, antimacrophage or antineutrophil serum had no effect on the numbers of schistosomula recovered from the lungs of immune animals, but in the mice treated with anti-eosinophil serum the numbers of schistosomula and adult worms recovered increased to the levels seen in normal non-immune animals. Furthermore, sera collected from the partially immune mice and passively transferred to uninfected mice conferred a marked resistance to infection as measured by recovery of schistosomula; this was also abrogated by treatment with antieosinophil serum. These studies suggest that antibody-dependent cell-mediated immunity to schistosomiasis occurs in vivo, and also establishes a role for the eosinophil in immune systems.

Presently, studies involve the profoundly immuno-suppressive effects of a metabolic disease (diabetes mellitus) and of an infectious protozoan disease (toxoplasmosis). In the former, streptozotocin-induced diabetes mellitus in mice resulted in the marked suppression of the host granulomatous reaction to schistosome eggs trapped in the liver, accompanied by amelioration of hepatosplenic disease. This observation has been followed by studies of the granulomatous response to schistosome eggs injected into the pulmonary microvasculature of mice with chemically induced (both streptozotocin and alloxan) and genetically determined diabetes mellitus. In all cases granuloma formation was suppressed and the amount of suppression was related to the degree of hyperglycemia. The suppressive effect of diabetes mellitus was reversed by treatment with insulin. Streptozotocin-induced diabetes mellitus was also associated with suppression of specific antigen-induced delayed footpad swelling and with significant prolongation of skin allografts across the H-2 area.

In addition to multiple other effects on the host-parasite relationship, infection with Toxoplasma gondii resulted in profound suppression of granuloma formation around S. mansoni eggs injected into the pulmonary micro-vasculature for at least 20 weeks and perhaps permanently.

B. Biology and Pathogenesis

AI-07453-10 G. R. DeFoliart (University of Wisconsin)

LaCrosse (LAC) virus was isolated from 3 suspensions of Aedes triseriatus larvae and from 1 suspension of male mosquitoes reared from larvae collected from basal tree-holes in Wisconsin during March, April, May, and June of 1973. Isolations of this virus were also obtained from 5 suspensions of A. triseriatus larvae and 4 suspensions of male mosquitoes originating from water in old automobile tires. Virus transmission to suckling mice was accomplished by female A. triseriatus reared from larvae collected from the tires.

Only two tree-holes and the tires yielded LAC virus-infected larvae and adults. The minimum field infection rate (MFIR) for these tree-holes and the tires, based on one infected larva per pool, was 1/110 larvae from the tree-holes and 1/20 larvae from the tires. The MFIR for adults 1/41 male mosquitoes originating from one of the tree-holes and 1/27 for male mosquitoes originating from the tires. The MFIR for female mosquitoes reared from larvae collected from the tires was 1/110. No virus was isolated from 255 female mosquitoes reared from larvae collected from the tree-holes. These findings strongly suggest that A. triseriatus is the reservoir of LAC virus and that transovarial transmission of the virus in A. triseriatus is the mechanism responsible for the survival of this arbovirus during the winter season in the north-central United States.

V. BIOCHEMISTRY AND PHYSIOLOGY BRANCH

The approximate level of support given the branch:

Type of Grant	Number	Amount
Research Grants[1/]	166	$9,152,072
Training Grants	1	32,834
Fellowships	29	377,032

Summary

The most interesting work supported by this branch is to be found in the mechanisms of resistance to antimicrobial agents research and its "offspring" --recombinant nucleic acid manipulations. Three meetings of the NIH Recombinant DNA Molecule Committee have taken place in the interim since our last annual report. Dr. Delappe has attended all of them and has witnessed the slow and, at times, painful and frustrating progress in the evolution of NIH guidelines governing research in this area.

As an interim measure the branch sent out in December, 1975, a modified version of Dr. Jacobs' letter requesting a signed pledge from each grantee in our program that he would adhere to interim guidelines more explicit than those of Asilomar governing recombinant nucleic acid experiments involving possible biohazards. We have had a 100 per cent response to our request.

In addition to the above interim measure, certain recombinant DNA research grants approved by both study section and council have been sent to the NIH Recombinant DNA Molecule Committee for its approval prior to issuance of a statement of grant awarded.

A new description of the responsibilities and interests of NIAID in the area of recombinant nucleic acids has been prepared for inclusion in the overall NIH referral guidelines.

Prior to the January, 1977 meeting of the National Advisory Council, a program in the recombinant nucleic acid area will be prepared for Council acceptance. This will include appropriate goals and content for the program.

1/ Includes 7 Career Awards ($173,262)

A. Methods for Other Diseases

1. Mechanisms of Resistance to Antimicrobial Agents (MRAA) (SEP)

During the past ten years, the importance to health problems of microbial resistance to therapeutic agents has become increasingly apparent. The relevance of this phenomenon to other interests of this Institute such as hospital-acquired infections of staphylococcal origin and investigations of the gonococcus is obvious. At this point, there is substantial clinical and epidemiological evidence that development of this resistance, and especially plasmid (extrachromosomal) mediated drug resistance, represents a major problem in medical care. Since most of the projects dealing with the development of drug resistance are of a basic nature and have been concerned with defining the molecular and biochemical basis for resistance, the Special Emphasis Program in this area has been transferred from the Bacterial and Mycotic Diseases Branch to the Biochemistry and Physiology Branch.

The principal goal of this Special Emphasis Program is to elucidate fundamental biological mechanisms involved in the development of drug resistance in microorganisms and to increase our basic understanding of this phenomenon. Specific goals of this Special Emphasis Program involve investigation of the origin, development, evolution, expression and mechanism of drug resistance in a variety of specific microorganisms. Examples of microorganisms of particular interest are (but not limited to): Enterobacteriaceae, Pseudomonas, Neisseria, staphylococci, streptococci, mycobacteria, mycoplasmas, and pathogenic fungi.

Projects of special interest to this program are included in one or more of the following major categories: (1) genetic and structural studies of R factors and related plasmids; (2) origin, development, and evolution of drug resistance in microorganisms; (3) replication and conjugal transfer of plasmids; (4) biochemistry and genetics of plasmid determined functions, especially resistance to antimicrobial agents; (5) correlated epidemiological and microbiological studies of naturally occurring plasmids with special reference to R factors.

Specific examples of research progress during the current fiscal year follow:

AI-12448-02 J. E. Davi (University of Wisconsin)

Dr. Davies has utilized antifungal compounds to identify new inhibitors of protein synthesis with which we might obtain resistant mutants. Several new inhibitors of eukaryotic protein synthesis have been found. Anthelmycin inhibits the puromycin release of nascent peptide chains from rat liver polysomes, thus it is a peptidyl transferase inhibitor. Ninfungin and alphasarcin are two small, basic proteins which inhibit elongation but not initiation of protein synthesis. They have much less effect on prokaryotic protein synthesizing systems. Ninfungin does not inhibit peptidyl transferase, amino-acyl tRNA binding, or EF-1 function. Alpha-sarcin inhibits EF-1 function, probably an enzymatic step. Hygromycin B is the first aminoglycoside to specifically inhibit eukaryotic protein synthesis. Its mechanism of action is undetermined but it complexes copper, cobalt, or nickel ions and this is necessary for its action in vivo but apparently not in vitro. The finding that it binds these ions suggests the proper configuration about the previously unassigned orthoester linkage in this molecule.

AI-09152-08 J. Strominger (Harvard University)

The overall objectives of the project are to describe the physiological functions of various penicillin-binding components, the nature of their interactions with penicillin and the relationship of these interactions to the killing of bacterial cells by penicillin. The goal for the present year was to study the penicillin-binding components of Staphylococcus aureus and Escherichia coli and to continue genetic studies in E. coli in an attempt to isolate mutants in which the various penicillin-binding components have been altered. The two penicillin-binding components of S. aureus have been isolated and attempts are now being made to separate them from each other. In addition, a third protein whose activity is inhibited by penicillin has been identified and it also has been isolated, although it does not interact covalently with penicillin. The latter enzyme has penicillinase, transpeptidase, and carboxypeptidase activities and is a very unusual protein.

Two D-alanine-carboxypeptidases have been purified to homogeneity from Escherichia coli. One of them is in the supernatant solution of a cell extract, and the other

is in the membrane fraction. The components in the membrane fraction bind penicillin covalently and in fact it contains two polypeptides, each of which binds penicillin, thus making them resistant to penicillin.

AI-12630-01 D. Nakada (University of Pittsburgh)

β-Lactamase (penicillinase) coded by a non-transferring plasmid (conferring ampicillin resistance) has been purified and characterized. Since the NTP 1-coded β-lactamase is exclusively localized in the periplasmic space, β-lactamase was released from the NTP 1 plasmid-carrying E. coli 18R405 cells by osmotic lysis, then purified by DEAE cellulose and phosphocellulose column chromatography. Electrophoresis of purified, radioactive β-lactamase in acrylamide-SDS gel showed a single band with an estimated molecular weight between 20,000 and 25,000.

Minicells containing NTP 1 plasmids were incubated with radioactive amino acids, and labeled proteins were analyzed by acrylamide-SDS gel electrophoresis. A protein with the same mobility as the purified authentic β-lactamase was detected as the major protein synthesized in minicells together with a few other minor proteins. Those minor proteins were also synthesized in minicells which did not contain NTP 1 plasmid. In an in vitro transcription-translation-coupled system from RNase I⁻ strain of E. coli, we have synthesized β-lactamase in the presence of NTP 1 DNA. β-Lactamase activity was detected by microiodometric assay. The major protein synthesized in this system in the presence of radioactive amino acids showed the same electrophoretic mobility in gel electrophoresis as the purified authentic β-lactamase. A few other minor proteins were also synthesized (not characterized yet).

AI-12500-01 J. R. Murphy (Harvard Medical School)

This worker has developed a general screening method for the detection of diphtheria tox gene mutants. He wanted to develop a rapid inexpensive assay for diphtheria toxigenicity, since he recognized early on during the search for the first of the Corynebacteriophage β tox regulatory mutants that all of the methods that were available for the assay of toxin were not sufficiently rapid or were too costly to screen the numbers of potential mutants that would be necessary to make the study of

diphtheria *tox* regulation feasible. The rationale that he used for such an assay was to employ a tissue culture cell line that would be sensitive to the effects of toxin, such that in the presence of small concentrations of toxin the cell line would not grow. Therefore, in the absence of active toxin the cell line would grow at its maximal rate and produce sufficient organic acids to turn a pH indicator from pink to yellow. In the presence of active toxin, the cell line would be inhibited and the pH indicator would remain pink.

The ability to assay either culture supernatants or agar plugs from β-plaques for toxigenicity by the CHO system in microtitre plates has allowed him to screen very large numbers of potential *tox* mutant lysogens of *C. diphtheriae*. He has been able to screen potential *tox* mutant lysogens of *C. diphtheriae* at a rate of greater than 1000 per day.

B. Recombinant Nucleic Acids and Genetic Engineering

Specific examples of research progress during the current fiscal year follow:

AI-10885-05 S. Falkow (University of Washington)

Dr. Falkow had been successful previously in isolating the plasmid sequences which encode for the heat stable (ST) enterotoxin of *E. coli* isolates from scouring calves. These gene sequences have been ligated to the plasmid ColE1. The protein sequences encoded by the ST gene sequences are currently under study in an *E. coli* "mini" cell system as well as a cell-free protein synthesizing system. During the past year, he has also been successful in isolating the specific sequences which encode for the heat labile (LT) enterotoxin of *E. coli* isolated from piglets. The plasmid encoding for LT, P307, is a 60×110^6 dalton plasmid. When treated with the restriction endonuclease, EcoRI, this plasmid is cleaved into some 11 distinct fragments ranging from 12×10^6 daltons to 0.8×10^6 daltons. Ligation of fully cleaved Ent P307 DNA to the cloning vehicle RSF2124 did not yield a hybrid plasmid capable of synthesizing the LT enterotoxin. He concluded, therefore, that the structural gene for LT contained a site cleaved by EcoRI that disrupted the synthesis of a functional LT enterotoxin protein. Subsequently, he subjected Ent P307 DNA to limited

digestion with the EcoRI enzyme to yield fragments containing contiguous sequences of DNA. Ligation of these partially digested fragments to RSF2124 permitted him to isolate a number of hybrid clones which, indeed, synthesized a functional LT enterotoxin as evidenced by a strongly positive Chinese Hamster Ovary tissue culture assay. It is now clear that the minimal number of contiguous fragments necessary for enterotoxin synthesis consist of a 4.4 x 10^6, 2.2 x 10^6 and 0.8 x 10^6 dalton EcoRI fragment. Each of these individual fragments have also been cloned into RSF2124 and we are currently screening them to determine if one (or more) of these fragments encodes for a polypeptide which can react immunologically with specific anti-LT serum. Such a clone would be a strong candidate for a vaccine strain. The composite LT + hybrid plasmid is also being analyzed with other restriction enzymes to determine if the entire LT sequence can be isolated on a DNA segment which can be isolated as an intact fragment. Such a fragment would be of considerable value in tracing the relationship between LT and ST gene sequences by DNA-DNA hybridization.

AI-08619-08 S. Cohen (Stanford University)

Although *in vivo* recombination between different bacterial plasmids has been well documented, little has been known about the functional interrelationships and mutual compatibility of separate and possibly distinct types of replication mechanisms that coexist on a single R-factor molecule. Using the EcoRI joining and transformation techniques they have constructed a plasmid (pSC134) that contains replicons having different replication characteristics. Investigations with this hybrid molecule have indicated that such plasmids constructed from two operationally competent and functionally distinct replicons can be maintained stably in bacterial cells. Moreover, the hybrid plasmid was shown to utilize the functionally distinct modes of replication as specified by both of its parents.

AI-07194-11 D. R. Helinski (University of California, San Diego)

A hybrid plasmid has been constructed *in vitro* from two EcoRI endonuclease generated fragments; one of 4.5 x 10^6 molecular weight that determines resistance to kanamycin, the second a self-replicating derivative of colicinogenic

plasmid E1 that has a molecular weight of 2.2×10^6. The recombinant plasmid was found to be able to replicate extensively in E. coli in medium containing chloramphenicol, facilitating the isolation of large quantities of plasmid DNA. The difference in size between the two fragments permitted their preparative separation by agarose gel electrophoresis. The purified kanamycin resistance fragment, incapable of self-replication, was used to select a single EcoRI endonuclease derived fragment from the sex factor F' lac. This fragment, exhibiting a molecular weight of about 6×10^6, carries the genes essential for maintenance of the F replicon in E. coli cells. The ability to obtain large quantities of the purified, non-self-replicating, kanamycin resistance fragments provides a useful method for the isolation of the replication region of bacterial replicons in general.

The studies during the past year have provided them with a clear understanding of the genetic and biochemical factors that are concerned with the duplication of circular DNA plasmid elements in bacteria. This is of significance in view of the importance of the R plasmids in the determination of antibiotic resistance in both pathogenic and nonpathogenic bacteria and the importance of various plasmid elements in pathogenic bacteria (e.g. enterotoxin plasmids) specifying toxin production. In addition, these observations on the replication of plasmid DNA molecules should provide further insight in our understanding of the replication of circular DNA tumor viruses. Finally the development of the cloning vehicle systems involving plasmid elements provide them with new approaches to the study of the molecular biology properties of viral agents and prokaryotic and eukaryotic genes of medical interest. Hopefully, in the not too distant future these systems will provide the means for the products of medical interest (for example, insulin, immunoglobulins).

COLLABORATIVE RESEARCH PROGRAMS

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Collaborative Research Programs

National Institute of Allergy and Infectious Diseases

Annual Report for FY 1976

Report of the Associate Director for Collaborative Research

During FY 1976 there were major changes within the NIAID having a significant effect on Collaborative Research programs. Dr. Richard M. Krause became Director of the NIAID on November 1, 1976 following the retirement of Dr. Dorland J. Davis on July 31. During the interim, Dr. John R. Seal served as Acting Institute Director and was subsequently appointed by Dr. Krause as Acting Deputy Director. In early January 1976, the staff was apprised of a proposed reorganization plan which would introduce a structure based on the Institute's two major scientific program areas, viz 1) Microbiology and Infectious Diseases and 2) Allergic and Immunologic Diseases. The funding mechanisms, i.e. grants and contracts, for extramural support of these two areas of research, were to be combined under a single management system. These organizational changes, designed to bring new operating efficiencies while at the same time more effectively articulating the Institutes purposes, contributions and potentials, are consonant with the recommendations of the NIH Program Mechanisms (Cooper) Committee, published in 1973.

Collaborative Research programs have been affected in a number of ways to date and it is anticipated that before the end of this calendar year, Collaborative Research will cease to exist as an organizational entity.

To summarize the current status

1. The Research Resources Branch has been given the added responsibility and staffing for the reagents and resources activities once assigned to the Transplantation and Immunology Branch. The Research Resources Branch has been relocated to the Westwood Building and has become organizationally a part of Extramural Operations. Dr. John E. Nutter is the Chief of the Branch and his staff includes Dr. John G. Ray, Mr. Donald B. Hare and Mrs. Sylvia Cunningham.

2. On the retirement of Dr. Donald E. Kayhoe as Chief of the Transplantation and Immunology Branch on April 1, 1976, Dr. Paul D. Pedersen was appointed Acting Chief. He is assisted in his responsibilities for continuing histocompatibility matching and antilymphocyte serum evaluation programs by Dr. Daniel I. Mullally. The Transplantation and Immunology Branch remains organizationally a part of Collaborative Research and has been physically relocated in the Westwood Building.

3. The Geographic Medicine Branch is still part of Collaborative Research and also has been moved to the Westwood Building. Dr. Earl S. Beck is Chief of the Branch and his staff of program officers consist of Dr. Kenneth O. Phifer (International Centers for Medical Research (ICMR), Viral Diseases, Parasitic Diseases), Dr. Richard E. Horton (Leprosy and Malnutrition),

Dr. Paul D. Lambert (Tuberculosis and Environmental), and Dr. Carl E. Miller (Cholera). Ms. Beatrice B. Benson the Administrative Assistant for Geographic Medicine Branch was detailed to the Laboratory of Clinical Investigation, Building 10 in March and Ms. Mary G. Lauer is serving as her replacement on an interim basis.

4. The Infectious Disease Branch is still physically located in Building 31 on the NIH Campus and organizationally has been assigned to the Office of the Acting Deputy Director. This arrangement was occasioned by the heavy involvement of the Branch in the new, highly visible and demanding National Influenza Program. Dr. George J. Galasso is Chief of the Branch and has been assigned special responsibility in assisting Dr. John R. Seal with the research efforts of the National Influenza Immunization Program. Key Branch staff include Dr. Franklin J. Tyeryar (Hepatitis and Influenza), Dr. Fred J. Payne (Antivirals and Clinical studies), Dr. James C. Hill (Bacterial Diseases), and Mrs. Martha J. Mattheis (Viral Respiratory Diseases, Vaccine Evaluation Centers and Investigational New Drug Application files which involve all studies using experimental vaccines or drugs).

5. The Associate Director for Collaborative Research, Dr. Howard A. Minners has remained in Building 31 on the NIH Campus, while Dr. Robert J. Byrne the Assistant Associate Director for Collaborative Research is located in the Westwood Building along with the Administrative Officer for Collaborative Research, Ms. Marilyn M. Page and her supporting staff.

6. The Contract Management Branch, which since 1973 has been in the B wing of Building 31 and organizationally under the Director (1973-75) and Executive Officer (1975-1976), is now located in the Westwood Building and has been incorporated into Extramural Operations. Mr. Merle J. Callahan is Chief of the Branch and assisted by Mr. Willard C. Hill, Mr. Jay W. Seering, Ms. Toni A. Abbaticchio and Ms. Mildred M. Applestein.

The effect of the above organizational changes and physical relocations have created a few expected stresses and strains but without serious breakdowns thus far. The physical separation of the Infectious Disease Branch from the Contract Management Branch has created problems at a time of peak workload.

Despite the continued heavy workload the staff has still been able to participate in a limited amount of training for themselves and to serve in the training of others. During FY 1976 Collaborative Research staff participated in the Extramural Program-Collaborative Retreats; Dr. James C. Hill, IDB, attended the University of Minnesota epidemiology course in the summer of 1975 and Dr. Franklin J. Tyeryar, IDB, attended the Workshop on New Advances in Tissue Culture at the Alton Jones Institute in March 1976. Dr. John E. Nutter continued as a member of the NIH Staff Training - Extramural Programs committee and a number of Collaborative Research scientific staff participated in selected STEP modules. Dr. Nutter also served as a STEP Module Director.

Three Grants Associates, Dr. Catherine Henley, Dr. Luiz Barbosa and Dr. George Steinberg received orientation and training in the Infectious Disease Branch. The Infectious Disease Branch also provided training for a Management Intern, Ms. Betty Lee.

Collaborative Staff were also involved in NIH committee activities pertaining to contract-sponsored research. Dr. Robert J. Byrne chairs a Subcommittee of the NIH Action Committee on Review of Research and Development Contract Proposals and Dr. George J. Galasso chaired the NIH Committee to Revise the Guide for Project Officers.

The Freedom of Information Act and the Privacy Act continue to place demands on staff mainly in attending NIH meetings, discussions and reviewing and interpreting new issuances. In practice, there has been only one freedom of information request during FY 1976 and this one case has involved considerable time and effort and continues to do so. The contract-related workload due to the Privacy Act implementation turned out to be considerably greater than was actually necessary in view of current interpretations of the Act as announced on May 17, 1976. We were first advised that the Privacy Act applied to practically all contracts involving human subjects, and this meant describing contractor systems of records and forwarding these descriptions for publication in the Federal Register. Later, after considerable internal debate and reluctance on the part of some contractors to comply with systems description, we were advised that only contracts in which we required retrieval by individual identifiers fell under the Act.

As in past years, the funding allocation for Collaborative Research programs varied considerably as the year progressed and was influenced by varying interpretations on the figure for the continuing resolution; whether we would receive the President's or Congressional budget, funds available and required for other objects and the amount required by and releasable from the Director's Reserve. Preliminary allocation for Collaborative Research contracts and agreements was $10,050,000 which turned out to be somewhat higher than is now projected for FY 1976 obligations. It is presently understood that FY 1976 funds can be carried over into what is called the Transition Quarter (July, August and September, 1976). Fiscal Year 1977 starts October 1, 1976 and based on preliminary OMB response the funding for combined grants and contracts appears somewhat improved over FY 1976. The Institute is expected to receive an additional $4,000,000 of no-year funds for contracts on influenza as part of the National Influenza Immunization Program which is a concerted effort to combat the threat of a possible pendemic of swine virus type influenza in 1976-1977.

The Director conducted Collaborative Research program reviews on a Branch by Branch basis in October 1975. Following these reviews he met with key staff and discussed Collaborative Research priorities for the remainder of FY 1976. These discussions were instrumental in determining new levels of support for individual programs and in some cases phasing out of projects and programs.

The implementation of an Institute contract information system, designed by Isabell Rosenberg and continuously updated, has been very useful in determining the current status of obligations and end of year projections as

well as in projecting commitment bases for the next two or three years.

The advisory system for NIAID's Collaborative Research programs presently consist of two committees, Infectious Disease Committee and the Transplantation and Immunology Committee, both of which were recently rechartered and given additional responsibilities including possible review of grant applications. The Collaborative Research's Geographic Medicine Programs have been served by advisory Panels constituted of consultants and government scientists knowledgeable in the areas of research relevant to the respective panel. The Research Resources Branch has continued to receive peer advice on an ad hoc basis or through established committees, such as the NIH Recombinant DNA Molecule Program Advisory Committee, as called for by the circumstances. The primary scientific-technical review of contract proposals has for the most part, been conducted by ad hoc groups, including representation from chartered committees or by U.S.-Japan Panels. The entire advisory system is now under review and revision to meet the Institute's new organizational structure. Extramural Operations has been designated as the administrative focal point for advisory systems serving the Institute's grant and contract sponsored research.

Inter-Institute and inter-agency coordination are critical to the success of Collaborative Research programs. In the case of influenza research, coordination has been recently carried out by a DHEW influenza group consisting of Dr. David J. Sencer, Center for Disease Control, Dr. Harry M. Meyer, Jr., Bureau of Biologics, Food and Drug Administration and Dr. John R. Seal, NIAID. The NIAID's influenza programs had been carefully coordinated from the beginnings with Institute staff involved in a variety of national and international activities including the influenza segment of the U.S. - U.S.S.R. Science Exchange Program. Coordination of NIH hepatitis research was formalized in the summer of 1975 with the establishment by the Deputy Director of NIH of the NIH Hepatitis Coordinating Committee, chaired by Dr. John Seal. Coordination is implemented by a subcommittee of scientists engaged in hepatitis research. This Subcommittee also called "Mini Committee" is chaired by Dr. Franklin J. Tyeryar and reports on a regular basis to the NIH Hepatitis Coordinating Committee. Coordination of the hepatitis research at the NIH is considerably improved from three - four years ago. There is also active coordination on interferon between the National Cancer Institute (NCI), National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), and the NIAID directed to the antiviral and antitumor effect of interferon.

Two U.S. Japan Program activities have required special coordination. The Malnutrition Program has involved the National Institute of Child Health and Human Development (NICHHD), NIAMDD as well as NIAID, with NIAID providing funds for contracts and the support of Panel meetings, conferences and site visits. Arrangements are being made with NIAMDD and NICHHD for NIAID to terminate its support of contract sponsored research as of the end of FY 1976. The U.S.-Japan Panel on Environmental Mutagenesis and Carcinogenesis has involved the National Institute of Environmental Health Sciences (NIEHS), NCI and the NIAID and while one of the Institute's staff serves as co-program officer, all U.S. funding comes from NIEHS.

Since the reports of the individual Branches highlight scientific progress and program development, it was decided that this final report of the Associate Director for Collaborative Research identify situations in which Collaborative Research programs were by design, responsive to national health and health research needs. In most cases, the outcome or payoff has not yet been realized, but the preparation, planning and the investment in time and money have most definitely enhanced chances for success in benefiting human health.

While not discussed in the report of the Infectious Disease Branch, the identification of a swine influenza-like virus as the cause of possibly 500 cases of human influenza at Fort Dix found the Institute uniquely prepared to participate in the National Influenza Immunization Program. The Institute's IDB had established an Influenza Study Center at Baylor University two years previously and this Center as well as other Institute-sponsored vaccine evaluation centers were prepared to conduct epidemiologic studies and to evaluate candidate influenza vaccines. Influenza research reagents, including specific reagents for swine influenza virus, were available through the Research Resources Branch and were quickly distributed to Institute contractors as well as researchers throughout the world. In essence, a structured Institute Influenza Program, consisting of inter-related contract projects was already in existance and ready to move. In a relatively short period of time it was possible to adjust contract priorities in order to focus on swine influenza and other possible pandemic strains of influenza virus and to step up the pace of vaccine evaluation in selected volunteer populations.

The Institute's mission in support of basic microbiology has included special efforts by grantee and intramural scientists in the area of DNA recombinant nucleic acid molecules. When the Asilomar Conference in February 1975 and the subsequent establishment of the NIH Recombinant DNA Molecule Program Advisory Committee focused scientific and public attention on nucleic acid recombinant biotechnology, the Institute asserted its role in present and future research. The respective roles of the National Institute of Allergy and Infectious Diseases and the National Institute of General Medical Science (NIGMS) were agreed to in a memo of understanding between the Directors of the two Institutes on March 12, 1976. One of the first priorities identified by the NIH Recombinant DNA Molecule Program Advisory Committee was the establishment of research resources for the construction and testing of safe vectors and hosts. Several contracts have already been awarded and it is anticipated that several more will be awarded in the Transition Quarter between FY 1976 and FY 1977.

Since its beginnings in 1964 the Transplantation and Immunology Program has been developing new knowledge and resources on the HLA surface antigens associated with lymphocytes. As a result there are now at least 37 antigens that can be typed for with the standard NIH tray and use of this tray is now an integral part of an evaluation study to determine the importance of HLA donor-recipient matching in the survival of transplanted organs, particularly cadaver-derived kidneys. The Institute-sponsored evaluation study on donor-recipient matching is the most extensive attempt of its kind and approximately 1,950 of the projected 2,400 study-patient accessions have

been obtained. There will be a two-year follow-up on each accession.

The last accession was obtained in February 1975 on an evaluation of the use of antibodies against thymocytes in reducing graft rejection by suppressing the recipients immune response. Both of these clinical trials, whose outcome could variously influence medical practice, were made possible by years of prior investments in reagents, tissue typing techniques, research on mechanisms and pathology of the immune response and research on immunosuppressive drugs and biologics.

The Geographic Medicine Program has been unique in having both contracts and grants as instruments of support. The value of this arrangement is illustrated by the Parasitology Program which is orchestrated by a Program Officer with advice from the U.S.-Japan Panel on Parasitology. The program is focused on two areas of research, schistosomiasis and filariasis. Since all NIH research grants on schistosomiasis and filariasis are identified with the NIAID's U.S.-Japan Program (R-22) there is thorough cognizance of the research by the Project Officer and contracts are designed to be and have been complementary to the grant-sponsored research. The narrative portion of the Geographic Medicine Branch report on parasitology provides specific illustrations of this complementarity.

The first report on Collaborative Research appeared in the Institute's Annual Report for 1962, and as presently envisioned this report will be the last for Collaborative Research as an organizational entity. While only two of the four branches reporting last year are still a part of Collaborative Research, it was decided to incorporate the reports of each Branch under the previous organizational framework because all four branches were organizationally part of Collaborative Research through May 1976.

TRANSPLANTATION AND IMMUNOLOGY BRANCH

The goals of the Transplantation and Immunology program are to develop and coordinate methods, procedures, and resources to enhance research in transplantation and immunology and to develop and utilize new methods for the evaluation and monitoring of such programs. While the program was instrumental in developing reagents and techniques for histocompatibility matching, the responsibility for this was recently transferred to the Research Resources Branch and is described in their section of the Annual Report.

I. Immune System and Disease - Tissue Transplantation

A. EVALUATION OF TISSUE MATCHING IN RENAL AND BONE MARROW TRANSPLANTATION

Although histocompatibility matching has been shown to improve the prognosis for success of living related kidney transplants, the data for cadaver kidney transplants have not been as conclusive. Studies which have tended to show an advantage for the well-matched cadaver kidney transplant have been reported by European centers, however, there has been no United States study which would be comparable to those done in Europe. There has been speculation that this may be due to the greater herterogeneity of the U.S. population and to lesser emphasis on transplantation of cadaver kidneys well matched for the HLA antigens. Since over 60% of all kidneys transplanted come from cadavers, it is important to determine the importance of HLA matching in cadaver kidney transplants. The investment made by this government in providing reagents, services, and patient care is substantial for each of the 3500 transplants done annually. Demonstration of the value of tissue matching is one means of justifying the expenditure of these funds. A collaborative study, recommended by the NIAID Transplantation and Immunology Advisory Committee, is under way with the objective of collecting complete and accurate clinical, laboratory, and other data on renal transplant patients to determine the efficacy of tissue matching.

At the present time, contracts with 16 regional transplant centers (involving 36 hospitals and 19 tissue typing laboratories) are resulting in collection of data on approximately 800 new transplants annually. In addition, there is a statistical data management center which is overseeing and coordinating the data collection and planning for the analysis of the data. All regional centers employ registrars who collect and submit data to the statistical center using forms devised particularly for this study. All laboratories in the study use standard procedures and participate in periodic quality control exercises so as to assure a continuing high level of proficiency in tissue typing techniques. Workshops have also been held for evaluation of technician performance and instruction in new techniques. The principal investigators in each of the contract institutions meet periodically as the Coordinating Committee and have assigned specific tasks to sub-committees on Laboratory Methods and Standards, Data Surveillance and Quality Control, and Data Analysis and Publication. The Policy Advisory Board for the study is convened periodically by the

Statistical Data Management Center to provide broad scientific oversight to the study. Since January 1, 1974, 1950 patients have been entered in the study. By the end of 1976, approximately 2400 kidney transplants will have been entered in the study. At the end of the follow-up period (minimum 2 years for all), this group of well-studied cases should provide a data base for analysis which is of statistical significance. Data analysis will not be done until after the end of the follow-up period. Some preliminary studies may be released prior to the end of the study.

Bone marrow transplantation has been shown to be a promising treatment for a number of hematologic, neoplastic and immunologic diseases. The key to clinical success lies in the control of the immunologic factors involved in rejection and in graft versus host disease in those cases in which marrow transplantation is successful. Techniques being utilized in these studies include HLA serotyping, genetic studies, mixed lymphocyte culture, cell-mediated lympholysis, and anti-globulin reactions. While most marrow transplants are performed between HLA identical, MLC non-reactive siblings, attempts are being made to identify marrow donors for patients who have no HLA identical siblings. In aplastic anemia 26 of 58 patients transplanted between Oct. 1970 and March 1975 are alive, with functioning grafts between 3 and 20 month after grafting. In leukemia, 11 of 30 patients receiving grafts from identical twins are in remission up to 5 years after grafting, while grafts from matched siblings show up to 31% (11 of 36) survival at one year and survival as long as 3.5 years.

B. ANTILYMPHOCYTE GLOBULIN PROGRAM

Program has sponsored a double-blind study of rabbit anti-human thymus globulin (RAHTG). One hundred cadaver kidney transplant recipients were treated with either RAHTG or a salt-poor human albumin placebo in a study which is designed to determine the efficacy of the RAHTG in preventing renal allograft rejection. The material used has demonstrated efficacy in prolonging skin and renal grafts in subhuman primates. This is a joint study with the Bethesda Naval Medical Research Program which developed the RAHTG and has the IND for its clinical evaluation, and the University of California, San Francisco where the clinical trial was conducted. The RAHTG used was developed with funds provided to the Navy by the TIB and animal testing was conducted at Bionetics Laboratories under an NIAID contract. All patients have been entered in the study and are now in the two-year follow-up period which will study long-term survivals and toxicity. This study is the first to use the double-blind technique to evaluate an ALG product with safety and efficacy proven in subhuman primate testing. The use of a single institution for the clinical trial limits the number of variables, while use of more facilities could introduce unexpected variables.

C. REGULATION OF IMMUNE RESPONSIVENESS

Programs described earlier (parts A & B) describe current efforts of this branch in the areas of histocompatibility matching and immunosuppression. During the past eleven years, the Transplantation and Immunology Branch contract program has been directed primarily towards immunosuppressive drugs and histocompatibility matching as means of prevention of or overcoming

graft rejection. Careful donor selection reduces the probability of introducing foreign antigens into the recipient and also reduces the incidence of graft rejection. Standardization of reagents (as in the NIAID Tissue Typing tray program) and improvement in organ exchange programs helps to provide better matched cadaveric kidneys for transplantation. Even with these advances, there are not enough well-matched kidneys available for transplantation and the problem of rejection related to incomplete matches still remains.

A third program is directed at regulation of immune responsiveness or "immunological tricks". This program was recommended by the Transplantation and Immunology Advisory Committee and consists of three contracts, each investigating a different aspect of the possible alteration of immune responsiveness. One contract is using the rat uterus, (an immunologically privileged site) as a model. It has been shown that pregnancy with a fetus bearing foreign antigens leads to the development of an apparently specific factor which prolongs survival of skin grafts with the same foreign antigens. A second contract is studying the mechanism by which activated T cells regulate the response of normal T cells to alloantigens. These cells have been shown to be either suppressors or stimulators. Suppressor cells have been found mainly in the spleen while stimulator cells have been found in the regional lymph nodes. A method to product a soluble suppressor factor in MLC supernates has been reported, however, it has not been characterized and its specificity is still being studied. The thrid contract is for the investigation of suppresion of cell-mediated immune response by serum alpha globulins. A low molecular weight alpha globulin has been purified to the 90% level and is capable of suppressing a MLC reaction. Larger amounts have been shown to suppress the immunological response of a mouse to specific stimulation. Much more work remains to be done in these three areas before any possible clinical applications will be apparent.

D. IMMUNOPATHOLOGY PROGRAM

Since a mojor cause for concern in renal transplantation is organ rejection, the Transplantation and Immunology Branch has since 1968 provided immunopathologic-diagnostic facilities for transplant programs throughout the United States. Renal tissue and/or sera from over 4500 patients have been submitted for immunopathologic study since the inception of this program. During the past year 559 renal and other organ biopsy specimens and 2075 serum samples from 955 patients were submitted for study. These materials came from 96 referring physicians plus 25 hospital laboratories requesting study of serum. Immunofluorescent, radio-immunoassay and elution studies are used to detect antibody, immunocomplexed and complement deposits in renal glomeruli and tubules. In addition, these specimens are tested for various etiologic agents responsible for diseases of the kidney. These procedures have contributed significantly to the understanding of kidney diseases in many nephritic patients throughout the country. The contractor has been able to differentiate immunopathologically the mechanism of graft failure, i.e. whether failure is due to a recurrent nephritis, the deposition of circulating immune complexes in the renal

glomeruli or tubules, or to an immunologic histoincompatibility reaction. This has facilitated the diagnosis and treatment of patients with renal failure.

For the past year, extensive efforts have been made to correlate the existing immunopathological data and the clinical status of the transplanted kidneys. It is expected that these studies should determine the usefulness of the various immunopathologic procedures in evaluating acceptance of the transplanted kidney as well as determining the nature of disease processes which may affect it.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Beathard, Gerald AI-52504 U. of Texas, Galveston $24,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all kidney transplants done at the University of Texas, Galveston. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began Jan. 1, 1974. During FY 1975 30 transplants were entered in the study from the participating hospital and tissue typing laboratory.
Braun, William AI-22536 Cleveland Clinic $28,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all kidney transplants done at the Cleveland Clinic. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began Jan. 1, 1974. During FY 1975 20 transplants were entered in the study by the participating hospital and tissue typing laboratory.
Carpenter, Charles AI-42552 Peter Bent Brigham Hospital $89,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all kidney transplants done at Peter Bent Brigham Hospital, Massachusetts General Hospital and Boston Children's Hospital. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began in September 1974. During FY 1975 85 transplants were entered in the study by the three participating hospitals. Tissue typing was done by two laboratories.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Dewitt, Charles AI-42507 University of Utah $13,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all kidney transplants done at the University of Utah Hospital and at the Salt Lake City VA Hospital. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 30 transplants were entered in the study from the two participating hospitals. One tissue typing laboratory serves both hospitals.
Flanigan, William AI-52511 U. of Arkansas $25,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Univ. of Arkansas Hospital and at the Little Rock VA Hospital. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 20 transplants were entered in the study from the two participating hospitals. Each hospital has its own tissue typing laboratory.
Henry, John AI-52513 SUNY, Syracuse $18,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Upstate Medical Center. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 40 transplants were entered in the study.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Homer, Louis D. AI-50001-01 Naval Medical Research Institute $401,000	Operation of a Statistical Data Management Center. Collection and analysis of data from the 16 centers supplying data under contract and 1 center not under contract on approximately 850 kidney transplants per year. Data collection began January 1, 1974 from most of the centers and in September and November of 1974 for those entering the study later. Data has been collected on nearly 2000 transplants.
Jonasson, Olga AI-22529 U. of Illinois $128,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the 7 hospitals which make up this center. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 125 transplants entered in the study from these hospitals. All tissue typing is done in a single typing laboratory.
Kano, Kyoichi AI-52514 SUNY, Buffalo $11,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at Buffalo General and Meyer Memorial Hospitals. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 20 transplants entered in the study from the two participating hospitals. A single tissue typing laboratory serves both hospitals.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Koontz, Samuel AI-42551 SUNY, Brooklyn $133,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Downstate Medical Center. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began in September 1974. During FY 1975 there were 100 transplants entered in the study by this center.
Lempert, Neil AI-52510 Albany Medical College	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Albany Medical College and at the Albany VA Hospital. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 30 transplants entered in the study from the two participating hospitals. One tissue typing laboratory serves both hospitals.
May, Allyn AI-22539 U. of Rochester $15,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Strong Memorial Hospital. Maintain quality control of matching procedures, freeze and store cells to allow subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 25 transplants entered in the study from this center.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Oleinick, Samuel AI-52512 U. of Oklahoma $6,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the University Hospital, Children's Hospital, and Oklahoma City VA Hospital. Maintain quality control of matching procedures, freeze and store cells for subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 10 transplants entered in the study from the three participating hospitals which are served by a single tissue typing laboratory.
Stastny, Peter AI-22531 U. of Texas, Dallas $40,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Parkland Memorial and Dallas VA Hospitals. Maintain quality control of matching procedures, freeze and store cells for subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 40 transplants entered in the study by the two participating hospitals. One tissue typing laboratory serves both hospitals.
Stein, Jay AI-52505 U. of Texas, San Antonio $30,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at Bexar County Hospital and at Wilford Hall Air Force Hospital. Maintain quality control of matching procedures, freeze and store cells for subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
	there were 30 transplants entered in the study by the two participating hospitals. Each facility has its own tissue typing laboratory.
Suki, Wadi AI-52506 Baylor College of Medicine $41,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the Methodist and Houston VA Hospitals. Maintain quality control of matching procedures, freeze and store cells for subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there was 15 transplants entered in the study from the two participating hospitals. One tissue typing laboratory serves both hospitals.
Thomas, E.D. AI-32511 U. of Washington $78,000	Evaluation of Tissue Typing Techniques in Bone Marrow Transplantation to determine the importance of histocompatibility matching. The importance of HLA and other histocompatibility antigens in the prevention of graft versus host disease and in the eventual outcome of the transplant is studied. Data to date indicate that histocompatibility identical matches with living related donors do better than mismatched bone marrow transplants. Also being investigated is the effect of transplantation on the immune responsiveness of the recipient.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - EVALUATION OF TISSUE MATCHING BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Yunis, Edmond AI-22530 U. of Minnesota $116,000	Evaluation of histocompatibility matching in renal transplantation. Provide clinical and laboratory data on all transplants done at the University, Hennepin County and Minneapolis VA Hospitals. Maintain quality control of matching procedures, freeze and store cells for subsequent immunologic studies. Data collection began January 1, 1974. During FY 1975 there were 160 transplants entered in the study by the participating hospitals which are served by a single tissue typing laboratory.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - ANTILYMPHOCYTE GLOBULIN BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Salvatierra, Oscar AI-22540 U. of California $44,000	Evaluation of RAHTG as an immunosuppressive agent. To collect and record clinical and laboratory information on patients on an RAHTG/placebo study, and to conduct special in vitro tests. This contract provides funds to collect laboratory and clinical data on the 100 patients in this double blind, stratified random study that is designed to determine if a program developed, RAHTG which is effective in subhuman primates, produces any significant improvement in the outcome of human cadaver kidney transplants. Under a Navy IND and protocols, the contractor has administered coded medications to patients. The Contract provides for funds for reporting the results of this study and for special laboratory tests to determine the patients immunologic status during the study. All 100 patients have been entered into the study at this time and a two year follow up period is underway, during which time clinical and laboratory findings will be sent to the Navy Statistical Center. Two years after the last patient was entered the code will be broken and the results analyzed. The information obtained will show the role that this preparation played in preventing allograft rejection.
Sell, Kenneth AI-10001-05 National Naval Medical Center $92,000	Clinical Evaluation of the Efficacy of Rabbit Antihuman Thymus Globulin. (RAHT). Collects and evaluates clinical and laboratory data on patients treated with antithymocyte globulin. The Naval Medical Research Unit has obtained a FDA-IND to study this product developed and produced by them with joint NIAID/NMRI funding. The material of demonstrated efficacy

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - ANTILYMPHOCYTE GLOBULIN BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
	in the prolongation of skin and renal graft survival in subhuman primates is being used in a double blinded clinical trial at the University of California at San Francisco. This Interagency Agreement provides funds for the collection and analysis of data on the clinical trial. All 100 patients have been entered on the study which is now in a two year follow up phase to determine late toxicity and efficacy.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - REGULATION OF IMMUNE RESPONSIVENESS BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Beer, Alan E. AI-42531 The U. of Texas, Dallas Health Science Center $10,000 (Est.)	A study of the mammalian uterus as an apparent immunologic privileged site. The contractor is to determine the cellular and humoral factors which control the uterus as an immunologically privileged site. Thus far, the contractor has been able to demonstrate specific prolongation of skin grafts and transplantation of ectoplacental cones beneath the renal capsule of multiparous female rats. Since this activity is specific, they are attempting to isolate and identify a serum component responsible for this in vivo phenomena. In a chimeric in vivo model of Fischer female rats repopulated with Lewis spleen and/or bone marrow cells, this contractor has shown that (1) the Lewis constituent of the chimera can be suppressed, (2) such potentially active cells can cross the placenta, and (3) these cells are released from suppression postpartum. Apparently, serum factors are likely mediators of this immunological suppression and can be further analyzed in GVH reaction reduction and capacity to prolong the lives of the offspring. These studies on in vivo models of the rat will be easiest to analyze for their specificity of reactivity.
Miller, Frederick AI-42533 Research Foundation of State U. of New York $15,000 (Est.)	Study the immunosuppressive effect of alphaglobulin fractions in an effort to selectively suppress the immune response by altering antigen recognition. Obtain these fractions in pure form from the most effective sources and determine by in vivo and in vitro assays the mode of suppressor activity. Thus far, fifty potential immunosuppressive types of human

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PROGRAM: TISSUE TRANSPLANTATION - REGULATION OF IMMUNE RESPONSIVENESS BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
	and animal material have been screened for activity and four extraction procedures have been employed for isolation of this activity. Five active immunosuppressive substances were found by *in vitro* MLC, induction of plaque forming cells, and by *in vivo* cell mediated immunity tests. These substances are: 1) low molecular weight material from human serum, 2) high molecular weight material from human serum, 3) bovine serum, 4) human placenta, and 5) human spleen. Approximately 90% pure product from low molecular weight human serum fraction has been obtained and it suppresses mouse immunological mechanism and an MLC reaction. These and other methods of purification are being applied to human placenta and spleen factors. Dose response curves for the various materials on MLC have been established and immunologic reconstruction experiments will be used to examine their modes of action.
Rich, Robert R. AI-42529 Baylor College of Medicine $10,000 (Est.)	A study of cellular mechanisms whereby activated T cells regulate the response of normal T cells to alloantigens. The contractor has determined that regulator T cells may either help or suppress the reactivity of responder cells in MLC. He has also shown that regulator cells can be activated by exposure to Con A or to alloantigens and that some T cells may modulate alloagressive reactions. Recent evidence indicates that cells which mediate the helper function in MLC reactions have different homing properties *in vivo* than those which suppress and suggest that these two functions are mediated by different sub-sets of T cells. Hopefully,

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
	these two cell types can be isolated and purified by receptor avidity for Con A or other techniques. This contractor also has some preliminary evidence that suppressor T cells in vitro release suppressive factors into the media. This activity is reasonably stable at freezer temperatures and enhances the possibility of purifying and characterizing the suppressor activity. Establishment of the CML assay with human and mouse cells opens the area of determining whether the population of activated suppressor T cells which regulate the proliferative T cell response in MLC also regulate directly or indirectly the development of killer cells.

Individual Contract and Agreement Tabulation

PROGRAM: TISSUE TRANSPLANTATION - IMMUNOPATHOLOGY BRANCH: TIB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Wilson, Curtis AI-42505 Scripps Clinic and Research Foundation $175,000	Study of the etiologic events and antigen-antibody systems involved in human glomerulonephritis and the course of nephritis in transplanted patients. To provide an immunopathological analysis of human kidney diseases by assessing serum and renal tissue before, during, and after transplantation. This service is provided to all U.S. Kidney Transplant Centers. Currently 96 such centers have participated in this service and significant contributions concerning the etiology and treatment of renal failure and graft rejection have been obtained.

CONTRACTOR PUBLICATIONS

Banowsky, L.H., Braun, W.E., Magnusson, M.O.: The medical and legal determination of death--its effect on cadaveric organ procurement. Journal of Legal Medicine 2:44, 1974.

Banowsky, L.H., Francis, J., Braun, W.E., Magnusson, M.O.: Lymphatic complications of renal transplantation. Urology 4:650, 1974.

Banowsky, L.H., Siegel, D.F., Hewitt, C.B., Stewart, B.H., Straffon, R.A., Magnusson, M.O., and Braun, W.E.: Increasing the supply of kidneys for transplantation: Use of donor organs with multiple vessels. Urology 4:643, 1974.

Banowsky, L.H., Montie, J.E., Braun, W.E., and Magnusson, M.O.: Prevention of wound infections in recipients of renal transplants. Urology 4:656, 1974.

Beer, A.E., Billingham, R.E. and Scott, J.R.: Immunogenetic aspects of implantation, placentation and feto-placental growth rates. Biology of Reproduction 12:176, 1975.

Beer, A.E.: Immunogenetic determinants of the size of the fetoplacental unit and their modus operandi. Europ. J. Obstet. Gynec. Reprod. Biol. 269:135, 1975.

Beer, A.E.: Fetal-maternal interrelations. Transpl. Proc. 7:893, 1975.

Beer, A.E. and Billingham, R.E.: Immunologic benefits and hazards in maternal-perinatal relationships. Ann. Int. Med. 83:865, 1975.

Beer, A.E., Scott, J.R. and Billingham, R.E.; Histoincompatibility and maternal immunological status as determinants of fetoplacental weight and litter size in rodents. J.E.M. 142:180, 1975.

Beer, A.E.,Head, J.R., Smith, W.G. and Billingham, R.E.: Some immunoregulatory aspects of pregnancy in rats. Transplantation (in press).

Beer, A.E., and Billingham, R.E.: 1976 Immunobiology of mammalian reproduction. In Foundations of Immunology series, Prentice Hall, pp. 1-233.

Belzer, F.O. and Salvatierra, O.: The organization of an effective cadaver renal transplantation program. Transpl. Pro. 6:93, 1974.

Belzer, F.O. and Salvatierra, O.: Cadaver kidney transplant failures at one month. N.Engl. J. Med. 292:535, 1975.

Belzer, F.O., Salvatierra, O., Cochrum, K. and Perkins, H.A.: Good kidney graft survival in hyperimmunized patients. Trans. Proc. (in press).

Border, W.A., Lehman, D.H., Egan, J.D., Sass, H.J., Glade, J.E., and Wilson, C.B.: Antitubular basement membrane antibodies in methicillin associated interstitial nephritis. N. Engl. J. Med. 291:381, 1974.

Braun, W.E., Banowsky, L.H., Straffon, R.A., Nakamoto, S., Kiser, W.S., Popowniak, K.L., Hewitt, C.B., Stewart, B.H., Zelch, J.V., Magalhaes, R.L., Lachance, J.G., and Manning, R.F.: Lymphoceles associated with renal transplantation: Report of 15 cases and review of the literature. Am. J. Med. 57:714, 1974.

Bruan, W.E., Crile, G., Jr., Straffon, R.A., Nakamoto, S., Isbister, W.H., Gifford, R., Deodhar, S., Khanna, R., and Banowsky, L.H.: Thymectomy and splenectomy in long-term (6 to 11 year) renal allograft recipients. Transplant. Proc. In press. 7:715 (Suppl.), 1975.

Braun, W.E., Strimlan, C.V., Straffon, R.A., Zachary, A.A., Bartee, S.L., and Grecek, D.R.: The association of W17 with familial renal cell carcinoma. Tissue Antigens. 6:101, 1975.

Buckner, C.D., Clift, R.A., Fefer, A., Lerner, K.G., Neiman, P.E., Storb, R. and Thomas, E.D.: Marrow transplantation for the treatment of acute leukemia using HL-A identical siblings. Transplant Proc. 6:365, 1974.

Callender, C.O., Simmons, R.L., Yunis, E.J., Toledo-Pereyra, L.H., De Shazo, B.S., and Najarian, J.S.: Anti-HL-A antibodies: Failure to correlate with renal allograft rejection. Surgery. 76:573, 1974.

Clift, R.A., Buckner, C.D., Fefer, A., Lerner, K.G., Neiman, P.E., Storb, R., Murphy, M., and Thomas, E.D.: Infectious complications of marrow transplantation. Transpl. Proc. 6:389, 1974.

Clift, R.A., Buckner, C.D., Williams, B., and Thomas, E.D.: Granulocyte transfusions in marrow transplant recipients. Chapter in: Leukocytes: Separation, Collection and Transfusion. J.M. Goldman and R.M. Lowenthal (Eds.) Academic Press, New York, 340, 1975.

Cochrum, K., Salvatierra, O. and Belzer, F.O.: The correlation between MLC stimulation and graft survival in living related and cadaver kidney transplants. Ann. Surg. 180:617, 1974.

Cochrum, K., Hoffman, P.M., Cullen, B.L., Belzer, F.O., Salvatierra, O., Fudenberg, H.H.: LIF production as an indicator of MLC reactivity. Clinical Research. 23:288, 1975.

Cochrum, K., Salvatierra, O., Perkins, H.A. and Belzer, F.O.: MLC testing in renal transplantation. Transpl. Proc. (in press).

Demling, R.H., Salvatierra, O., and Belzer, F.O.: Intestinal necrosis and perforation after renal transplantation. Arch. Surg. 110:251,1975.

Dixon, F.J., and Wilson, C.B.: Recent advances in immunology of glomerulonephritis. Proc. VIth Int. Cong. Neph. In press.

Dvorak, K.J., Braun, W.E., Stowe, N.T., Magnusson, M.O., and Banowsky, L,H.: Effect of high doses of methylprednisolone on the isolated, perfused kidney. Transplantation. 21:149, 1976.

Fefer, A., Mickelson, E. and Thomas, E.D.: Leukaemia antigens: Mixed leucoctye culture tests on twelve leukaemic patients with identical twins. Clin. Exp. Immunol. 18:237, 1974.

Glucksberg, H., Storb, R., Fefer, A., Buckner, C.D., Neiman, P.E., Clift, R.A., Lerner, K.G. and Thomas, E.D.: Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A matched sibling donors. Transplantation. 18:295, 1974.

Kano, K., Loza, V., Gerbasi, J.R., and Milgrom, F.: Studies on heterophile antibodies in transplantation sera. Transplantation. 19:20, 1975.

Lehman, D.H., Wilson, C.B., and Dixon, F.J.: Extraglomerular immunoglobulin deposits in human nephritis. Am J. Med. 58:765, 1975.

Loening, S., Banowsky, L.H., Braun, W.E., and Magnusson, M.O.: Bladder neck contracture and urethral stricture as a complication of renal transplantation. J. Urol. 114:688, 1975.

Magalhaes, R.L., Braun, W.E., Straffon, R.A., Hoffman, G.C., Shainoff, J.R., Osborne, G., and Deodhar, S.D.: Microangiopathic hemolytic anemia (MAHA) in renal allotransplantation. Am. J. Med. 58:862, 1975.

Matas, A.J., Simmons, R.L., Kjellstrand, C.M., Buselmeier, T.J., and Najarian, J.S.: Transplantation of the aging kidney. Transplantation. 21:160, 1976.

McCoy, G.C., Loening, S., Braun, W.E., Magnusson, M.O., Banowsky, L.H., and McHenry, M.C.: The fate of cadaver renal allografts contaminated before transplantation. Transplantation. 20:467, 1975.

Miller, F.: Serum Derived Immunosuppressive Substances I. Partial Purification and Range of Action. Transplantation. 21:179, 1976.

Potter, D.E., Holliday, M.A., Wilson, C.J., Salvatierra, O., Belzer, F.O.: Alternate day steroids in children after renal transplantation. Transpl. Proc. 7:79, 1975.

Rich, S.S., and Rich, R.R.: Regulatory Mechanisms in cell-mediated immune responses. J. Exp. Med. 142:1391, 1975.

Salvatierra, O., and Belzer, F.O.: Pediatric cadaver kidneys: their use in renal transplantation. Arch. Surg. 110:181, 1975.

Salvatierra, O., Wolfson, M., Cochrum, K., Amend, W., Belzer, F.O.: End-stage polycystic kidney disease: management by renal transplantation and selective use of preliminary nephrectomy. J. Urol. (in press).

Salvatierra, O., Potter, D., Cochrum, K., Amend, W., Duca, R., Johnson, R., Belzer, F.O.: Improved patient survival in renal transplantation. Surgery (in press).

Salvatierra, O.: Increased reliance on cadaver grafts for renal transplantation: considerations for optimum effectiveness. Transpl. Proc. (in press).

Scott, J.R., Beer, A.E. and Stastny, P.: The relationship of erythrocyte histocompatibility and Y dependent antigens to pre-eclampsia and eclampsia. J.A.M.A. (in press).

Scott, J.R. and Beer, A.E.: Immunological aspects of pre-eclampsia. Am. J. OB-Gyn (in press).

Storb, R., Thomas, E.D., Buckner, C., Clift, R.A., Fefer, A., Glucksberg, H. and Neiman, P.E.: Transplantation of bone marrow in refractory marrow failure and neoplastic diseases. AM J. Clin. Pathol. 62:212, 1974.

Strom, T.B., Tilney, N.L., Carpenter, C.B., and Busch, G.D.: Identity and cytotoxic capacity of cells infiltrating renal allografts. N. Engl. J. Med. 292:1257, 1975.

Theofilopoulos, A.N., Wilson, C.B., and Dixon, F.J.: The Raji cell radioimmune assay for detecting immune complexes in human sera. J. Clin. Invest. (in press).

Weiden, P.L., Storb, R., Kolb, H.J., Graham, T.C., Kao, G., and Thomas, E.D.: Effect of time on sensitization to hemopoietic grafts by preceding blood tranfusion. Transplantation. 19:240, 1975.

Weiden, P.L., Thomas, E.D., Storb, R., Fefer, A., Neiman, P.E., Clift, R.A., Buckner, C.D., and Lerner, K.G.: Marrow transplantation in aplastic anemia and leukemia. Comprehensive Therapy. 2:57, 1976.

GEOGRAPHIC MEDICINE BRANCH

In the Geographic Medicine Branch two international biomedical programs, the U.S.-Japan Cooperative Medical Science Program and the International Centers for Medical Research, operate under Public Law 86-610, the International Research Act of 1960.

The U.S.-Japan Cooperative Medical Science Program was the product of a January 1965 meeting of the Prime Minister of Japan and the President of the United States. The focus of the Program was to be on improving the health of developing countries, particularly upon health problems of recognized importance in Asia. Initially six categories were included in the Program: 1. cholera, 2. leprosy, 3. malnutrition, 4. parasitic diseases (schistosomiasis and filariasis), 5. tuberculosis, and 6. selected viral diseases. In 1972, a seventh category, environmental mutagenesis and carcinogenesis was added with contract and grant support from the National Institute of Environmental Health Sciences. Since grant and contract support is not derived from NIAID, funding data for this category will not be included in this report. Information on environmental mutagenesis and carcinogenesis workshops and conferences are included since these are sponsored by NIAID.

The International Centers for Medical Research Program consists of four United States universities, each with an overseas component. This Program is grant supported with the bulk of the funds utilized for research at the foreign site. The four current grants were renewed for an additional five years in FY75.

I. Prevention, Treatment and Diagnosis of Infectious Diseases U.S.-Japan Programs

A. CHOLERA

The evaluation of immunizing agents against experimental cholera are continuing since the field trial two years ago demonstrated only transient protection from circulating antitoxin. Emphasis is being placed on comparison of the protective roles of serum antibody and antibody elaborated by plasma cells in the intestinal lamina propria. The effectiveness of oral or parenteral toxoid in eliciting systemic and local intestinal immune responses is being studied in animals (Contract No. NO1-AI-0-0251). The results indicate a clear superiority of a combined immunizing regimen employing a single parenteral dose followed in four weeks by ten daily oral booster doses of toxoid. Dogs so immunized averaged 85% protection against live vibrio challenge for four months and about 50% protection for eight to thirteen months, compared to uninoculated controls. Serum antibodies appeared to play no significant role in such protection. In contrast, dogs given two subcutaneous (SC) toxoid injections were protected for only a few weeks, while serum antitoxin titers were high, and dogs given two oral immunization series were unprotected. There has been no test of dogs given a single parenteral dose SC followed

in four weeks by ten daily SC doses of toxoid. The SC-oral immunizing sequence required that the oral booster be divided, since no protection was seen after SC priming and a single oral booster. Protection in these studies appeared to be entirely antitoxic since the toxoid elicited only small and transient rises in serum vibriocidal antibody and SC immunization with commercial whole cell vaccine elicited 80-fold increases in vibriocidal antibody without producing significant protection. These studies strongly suggest that in dogs a combined parenteral-oral sequence gives longer lasting protection than seen after parenteral or oral immunization alone.

Evidence has also been obtained indicating that protection which follows repeated parenteral administration of toxoid is due solely to the protective role of serum antibody. Analysis of studies of dogs immunized parenterally with purified toxin or toxoid reveal a close linear relation between pre-challenge antitoxin titer and challenge outcome. Dogs passively immunized by transfusion of serum IgG antitoxin showed the same relationship between serum antitoxin titer and protection. Finally, dogs immunized SC with a formalinized toxoid showed no antitoxin-containing cells in jejunal lamina propria two weeks after immunization, when serum antitoxin titers and protection against challenge were high. Conversely, evidence also suggests that SC-oral immunization protects by stimulating local antitoxin production.

The protection caused by sequential SC-oral toxoid appears to come from more effective stimulation of the local intestinal immune mechanism than occurs after parenteral immunization alone. The mechanism by which protection after SC-oral immunization is prolonged is not clear and requires further study. Further study is also needed to determine the simplest effective oral booster regimen and to examine the important question of the role of possible adjuvants in enhancing the local immune response to orally administered toxoid. Some preliminary studies with orally administered toxoid in human volunteers have been initiated.

Recent findings in cholera patients in Bangladesh revealed a much larger volume of stool from malnourished individuals compared to better nourished individuals. The volume of stool per unit of time did not increase, but the duration of purging was as much as 50% longer. It is postulated this prolonged purging may be due to a slower replacement of mucosal cells in the poorly nourished individual.

The coordination of laboratory procedures and serotyping systems for characterizing noncholera vibrios being fostered by the United States and Japanese Cholera Panels is progressing well. All type-cultures in the Japanese collection have been sent to the United States, and the U.S. cultures are all in Japan. Cross checking of the U.S. serologic methods has reduced the number of vibrio serotypes from 100 to 79. New typing sera are being prepared by the contractor at Jefferson Medical College (Contract No. NO1-AI-3-2502). These new sera will be shared with the cooperating U.S. laboratory at CDC for final confirmation before being sent to Japan. With the exchange of the prototype sera in addition to the cultures, the two countries should be able to establish a single serotyping system in place of the two systems now in use.

The studies projected over a three-year period on the epidemiology of diarrheas caused by enterotoxin-producing E. coli are in their second year. It is becoming apparent that enterotoxin-producing E. coli play a highly variable role in their association with acute diarrheal illness. Fortunately, the Contractors (NO1-AI-4-2548 and NO1-AI-4-2549) are studying several populations with vastly different age, social, economic, and ethnic backgrounds. The pediatric studies in Brazil (Contract NO1-AI-4-2548) revealed nearly 50% of the patients harbored lactose fermenting organisms that produced cholera-like, heat-labile toxin (LT) as the only fecal pathogens. None of the matched controls had similar fecal pathogens. In the study of adults in Brazil, there were many patients (67%) with postural signs or symptoms of significant hypovolemia. However, nearly half of the 67% had an inflammatory process. Information from their matched controls is incomplete at this time. About one-third of the college students surveyed in Virginia (Contract NO1-AI-4-2548) had enterotoxin-producing E. coli in their stool. The number of students with an elevated serum anti-LT antibody was about the same in the matched controls as in the patients. The study in Navajo Indians (Contract NO1-AI-4-2548) is finding most GI illness is due to Shigella as the major cause of illness. These studies are continuing along with a U.S. family study. Also, there is some indication of heat-stable toxin (ST)-producing E. coli being associated with diarrhea in the rural population being studied in Kentucky (Contract NO1-AI-4-2549).

B. LEPROSY

Work on the in vitro cultivation of Mycobacterium leprae continued during the past year. Drs. O. K. Skinsnes and E. Matsuo, University of Hawaii, (Grant AI-10034) published preliminary results on their successful attempts to cultivate M. leprae on hyaluronic acid based medium (LA-3P). According to the results of these investigators, LA-3P artificial medium supports satisfactory growth of M. leprae within two to three weeks after inoculation. Further studies are in progress to enhance the basic growth requirements of the leprosy bacillus. This major breakthrough in the in vitro cultivation of M. leprae has tremendous potential as a source of tissue-free M. leprae for future immunochemical studies and immunological characterization of the antigen components of the organism. These studies are still preliminary and other investigators are working to confirm them.

In July, 1975, an Intraagency Agreement (YO1-AI-60001) was initiated with the U.S.P.H.S. Hospital at Carville, Louisiana. The purpose of this project was the establishment of a colony of nine-banded armadillos infected with Mycobacterium leprae. Dr. Waldemar Kirchheimer, project director for this project, has made excellent progress toward achieving this specific commitment. Up to the present time a screening operation has demonstrated that all armadillos caught in the wild are free of mycobacteriosis and have been judged disease-free and acceptable for admission into the colony at Carville. It is anticipated this valuable resource of M. leprae will eventually meet the Program's future requirements for large quantities of M. leprae for biochemical purification studies and perhaps serve as a useful reagent in preparing soluble skin testing antigen for clinical investigations.

Through grant support (AI-10927 and AI-11532), Dr. Eleanor Storrs at the Gulf South Research Institute has continued studies on the metabolic disposition of DDS (4, 4'diaminodiphenyl sulfone) and DADDS 4, 4'-diacetamidodiphenyl sulfone) in the armadillo model. It has been demonstrated DDS is acetylated and DADDS hydrolyzed in a manner similar to man. Using the armadillo, it was observed that repository DADDS produces relatively constant levels of DDS in plasma for prolonged periods of time. The observation is similar to the metabolic process in man. In addition, dose response studies are in progress to determine the prophylactic effect of DDS after inoculation of the armadillo with quantified numbers of M. leprae. In another group of experiments Dr. Storrs is attemtping to evaluate the therapeutic effects of DDS in armadillos with M. leprae disseminated infection. At this time it is difficult to anticipate how much of the knowledge derived from the armadillo model can be extrapolated to the chemotherapy approach of leprosy in man.

The Contract (NO1-AI-0-2283) with Stanford Research Institute has continued basic pharmalogical work on procedures for measuring antileprosy drugs in biological systems and in the application of these procedures to studies directed toward a better understanding of the treatment of leprosy with drugs. Dr. Jack Peters, project director for the Contract has demonstrated that the drugs like rifampin (RFM), clofazimine, and thalidomide do not interfere with chemical procedures that are used for determining dapsone (DDS) or its acetylated derivatives in biological fluids. Also, a high-pressure column chromatographic procedure has been developed for the measurement of RFM and desacetyl RFM in plasma that is accurate and sensitive to 0.1 µg of the compounds per ml. DDS and other sulfones do not interfere with this technique.

In a second group of experiments, the characteristics of acetylation and DDS disposition in South Indian subjects has now been completed. As in earlier population groups studied, it was found that the subjects acetylated DDS polymorphically in parallel with sulfamethazine, and exhibited a broad range of clearance rates of DDS that were unrelated to acetylator phenotype. In another study it was demonstrated there was no differences in the characteristics of DDS disposition in Costa Rican lepromatous patients who exhibited DDS-sensitive or DDS-resistant leprosy. In studies of the disposition of sulfoxone and solasulfone (early prototypes of sulfone) in leprosy patients in the PHS Hospital, Carville, LA, the SRI group found that, on regular therapy with these drugs, plasma levels of DDS were in excess of the Minimum Inhibitory Concentration (MIC) of DDS for Mycobacterium leprae. It can be postulated that only on interrupted schedules of therapy with these sulfones would patients be at risk for the emergence of DDS-resistant organisms.

In collaborative studies, the SRI workers have made numerous measurements of dietary and plasma levels of DDS in mice for the testing of the viability of M. leprae from patients. In this work, they established that the Minimum Inhibitory Concentration (MIC) of DDS for M. leprae in mice was approximately 3 mg/ml of plasma.

Under grant support to Stanford Research Institute (AI-00111), the following information has accrued: Comparative studies were performed on the disappearance rates of DDS [illegible] skin and sciatic nerve of male Lewis rats receiving 1.0 mg DDS intraperitoneally for four days. Decay times from the tissues were significantly longer than the plasma disappearance rate of 4.1 hours; they ranged from 5.2 hours for muscle to 6.8 hours for skin.

Dr. Louis Levy's studies at the Leprosy Research Unit at the U.S.P.H.S. Hospital, San Francisco (IAA-Y01-10004) have continued during the past year. Considerable progress has been made toward defining the activity against Mycobacterium leprae of a series of substituted 2, 4-diaminoquinazolines, a class of non-toxic inhibitors of mycobacterial dihydrofolate reductase potentially of great value in leprosy chemotherapy. Studies designed to measure the MIC of several of these compounds against M. leprae in mice and to demonstrate synergism with dapsone, were completed. The two most interesting compounds---5, 6-dimethyl-2, 4-diaminoquinazoline and 5-methyl-6-n-propyl-2, 4-diaminoquinazoline---were active when administered in concentrations as small as 0.0003 g %, and the activity of these compounds in concentrations of 0.001 to 0.01 g % combined with dapsone 0.0001 g % was greater than that of dapsone alone.

A new contract was awarded to Yeshiva University during the last quarter of FY76. The purpose of the contract is to provide information on immunological parameters which can be measured in in vitro to clarify whether "Transfer Factor" has efficacy and immunological specificity in the immunotherapy of lepromatous leprosy. Methods to enumerate T-cells, B-cells and monocytes in peripheral blood will be employed. Tests for cell-mediated responsiveness to specific antigens, i.e. lepromins and coccidioidin, as well as non-specific mitogens, (i.e. Phytohemoagglutinin, PHA) are to be included in these studies. Other assays proposed, include blastogenesis, LTF assay and the virus plaque assay for T-cells.

It is anticipated that this new contract will provide the necessary laboratory support for the controlled clinical trials to determine the role and specificity of "Transfer Factor" in treating leprosy patients at the Staten Island U.S.P.H.S. Hospital and the U.S.P.H.S. Hospital in Carville, Louisiana.

Studies of leukotaxis in patients with lepromatous leprosy have been expanded by Dr. Ward Bullock, Principal Investigator of Grant AI-10094. The sera of 19 patients with lepromatous leprosy were screened for the presence of proteins similar to a "chemotactic factor inhibitory substance" (CFI) found in normal human serum at concentrations too low to be determined without special techniques of serum concentration.

Incubation of the bacterial chemotactic factor with normal human serum resulted in 15% reduction of the chemotactic activity. Conversely, inhibitory activity causing 35% (range 35-94%) reduction of the bacterial chemotactic factor was found in the sera from 14 to 19 patients with leprosy. Although exceptions were noted, a correlation was found between the presence of the inhibitor and depression of delayed skin reactivity to a series of test antigens.

Biochemically, the chemotactic inhibitor in leprosy sera is indistinguishable from the CFI activity present in high concentrations in sera of patients of Hodgkin's disease. In the latter case, the inactivator is inhibitory to chemotaxis of mononuclear cells as well as polymorphonuclear cells. While the effect of leprosy sera upon monocyte migration has not yet been tested, it may be responsible for some of the defects in reactions involving CMI as well as inflammatory reactions to "nonspecific" tissue injury. The report of this work is now ready for publication.

C. MALNUTRITION

Among the various highlights of the past year was the initiation of a new contract with the University of California, Los Angeles (NO1-HD-5-2851). This study under the direction of Dr. Charlotte G. Neumann will be conducted in Nairobi, Kenya. The purpose of the project is to define the effects and interrelationships of intrauterine malnutrition and the developing immune system, particularly cell-mediated immunity and the possible role of an immunologic defect on the infant's subsequent response to measles immunization. The contract has not been in effect long enough to report data.

Excellent progress has been made in studies conducted by Drs. Michael Katz and Gerald Keusch (Contract NO1-HD-4-2859 with Columbia University). The object of this study is the determination of immunological capacity of malnourished individuals to respond to infection. The population of a Guatemalan highland Indian village, Santa Maria Cauque was chosen, because it had been under continuous surveillance by the Institute of Nutrition of Central America and Panama for a decade.

No abnormalities of function of the cord white blood cells was found with respect to their ability to ingest and kill bacteria. However, serum factors required for this white blood cell function were deficient in the low and intermediate birth weight group children. Indirect experimental evidence suggests that the deficient factor is serum complement. It appears that the degree of observed abnormality (i.e. deficiency of the serum factors) is greater in these studies than would be expected in comparable birth weight infants in populations of higher socio-economic status, who are therefore better nourished. This abnormality may be a basis for greater susceptibility to infection of the lower birth weight infants in this malnourished population.

The second part of this project concerned itself with specific responses of village children to measles infection. Measles has been a serious and often devastating infectious disease among malnourished populations. These studies evaluated antibody response to the natural infection and to live, attenuated measles virus vaccine. Further studies exploring the findings in both parts of this project are now in progress.

Work on protein malnutrition and prenatal alteration of the primate brain (squirrel monkeys) has continued at the Yerkes Regional Primate Research Center. These studies are directed by Dr. Sohan L. Manocha, Grant HD-06087.

In cytochemical and biochemical studies of the brain, significant changes were observed in the behavior of the lipofuscin pigments. The malnourished adult animals showed characteristic intracellular aggregations with enhanced lysosmal enzymes as compared to controls. When those animals were rehabilitated on balanced diets, the pigment accumulation along with enzyme levels greatly decreased in the neurons, while at the same time, perineuronal glia were found to pick up the lipofuscin bodies. In the developing fetuses and neonates, the lipofuscin bodies are observed in the malnourished animals at a much earlier age compared to the controls. The healthy neonates do not show any lipofuscin bodies in the motor neurons of the spinal cord, cerebellum and, to a great extent, cerebral cortex; whereas the 110 and 140 days-old fetuses from malnourished mothers reveal a growing number of lipofuscin bodies, the accumulation of which greatly increases as the animal grows. It appears that protein malnutrition in the developing period, among other functional disorders, has the impact of hastening the aging process as revealed by the quantities of "wear and tear" pigment in the nervous system. Concomitantly, changes have been observed in the activity of important enzymes related to neurotransmission, e.g. monoamine oxidase and acetylcholinesterase and the enzymes belonging to the TCA cycle, pentose shunt and gylcolytic metabolic pathway. Significant observations have been made on the activity of the secretary neurons which are being pursued. In addition, biochemical studies were carried out in order to quantitate the amount of DNA, RNA, protein and cholesterol in the nervous tissue of developing fetuses and neonates. The anatomical and histopathological studies are done at the light and electron microscopic levels.

The contract with St. Joseph Hospital (NO1-HD-2816) has made good progress in all areas during the past year. This project, under the direction of Dr. David Coursin was designed to further extend the basic research on the development of a new means of stimulating and recording electrophysiological responses in human subjects in field studies and providing for the automatic measurement of analysis of these data. Further support permitted the validation of the data that was stored on computer magnetic tape as well as making possible the comparison of the quality of the information required by the system with simultaneous recording on an Ampex FR-1800. Current work has resulted in the establishment of a new methodology for the study of large numbers of subjects with the information stored in a fashion that will allow for sophisticated computer analysis.

In addition, further progress has been made in improving the portability of the system in streamlining the operational relationships between field activities of acquisition of data and their analyses at the contractor's facilities. Based on this current status, Dr. Coursin is now submitting a grant proposal to NIH for the study of electrophysiological parameters in malnourished populations in Guatemala and Mexico. It is hoped that these studies will provide comprehensive new information on this important aspect of the impact of malnutrition on brain development and function.

D. PARASITIC DISEASES

Parasitic Diseases research is confined to two parasitic infections of very great importance in Asian countries---schistosomiasis and filariasis. NIAID's grant program on these two diseases is dependent to a great degree upon two Branch supported supply contracts. Investigators are supplied with animals infected with various species of schistosomes or filariids. These contracts have enabled investigators from diverse disciplines to initiate research programs designed to attack many basic questions yet unanswered. Research highlights of the past year from the work of some of these investigators is summarized in this report.

Schistosomiasis

During the past year, the schistosomiasis supply contract at the University of Michigan (NO1-AI-4-2504) filled 222 requests for 33 investigators. Of the 22 NIAID grantees working on schistosomiasis, 19 used the contract last year. One of the major objectives of this contract is to stimulate new research on schistosomiasis by making infected animals available; the past year 11 investigators utilized the contract for the first time.

Last year, one of our contractors (NO1-AI-2-2525) established a cell line from embryos of the snail host, Biomphalaria glabrata. This year, another contractor (NO1-AI-2-2527) developed preservation techniques whereby snail cells can be successfully frozen in 10% dimethylsulfoxide using insulated ampules transferred from room temperature to minus 16°C, to minus 86°C, and to minus 196°C at approximately one-hour intervals. Ampules of cells are currently stored in liquid nitrogen and are available for other investigators upon request.

An isothiocyanate derivative developed under an existing grant (Bueding--AI-08022) has been shown to have schistosomicidal properties in vivo when administered as a single oral dose to experimental animals. A most unusual property was provided by the observation that, in contrast to any other known antischistosomal compounds, three geographic strains of Schistosoma japonicum (supplied by Contract NO1-AI-4-2504) were at least as, if not more, susceptible to this drug than Schistosoma mansoni. Furthermore, this isocyanate derivative failed to exhibit any detectable mutagenic activity when tested in the absence or presence of liver microsome preparations with two highly sensitive Salmonella tester strains. This is of considerable interest, because all antischistosomal drugs presently in use are potent mutagens, and most of them have been shown to be carcinogens in laboratory animals.

The interrelationships among S. mansoni infection, immune responses and eosinophils continue to receive major emphasis by Dr. Colley under Grant AI-11289. Lymphokine eosinophil stimulation promoter (ESP) has now been shown to be produced by T lymphocytes, confirming a relationship between ESP and cell-mediated immune responses. In related grant studies, Warren (AI-08163) has initiated experiments in the site, timing and mechanism of killing. Preliminary data indicate that the most probable site of killing is

the skin and that destruction of the schistosomula occurs within the first three days after infection.

Studies on the biocontrol of schistosomiasis have shown (Lie--Grant AI-08520) that B. glabrata snails become sensitized and develop a reproducible, acquired, highly specific resistance when infected with irradiated Echinostoma lindoense sporocysts. Tissue reactions, including amebocyte capsule formation in the ventricle have been described.

Filariasis

Our filariasis service contract at the University of Georgia (AI-52519) continued to supply investigators from a variety of disciplines with infected animals and vectors for their research. One hundred and thirty requests were filled for 28 investigators representing 24 institutions. Seven of the nine currently funded NIAID grantees working on filariasis utilized this contract.

Considerable progress has been made during the past year's studies on the development and evaluation of model systems. Ash (Grant AI-10250) demonstrated the completion of the life cycle of Wuchereria bancrofti in a non-human primate host. This finding highlighted the possible utility of monkeys as hosts for W. bancrofti, an infection that had been believed to be restricted to man.

In grant-supported studies of cytoplasmic incompatibility for filariasis control (Barr--Grant AI-11847), it was shown that reproduction in Culex pipiens is controlled by a mutualistic symbiont which occurs in the gonads of males and females and which is transmitted transovarially to all individuals. The mutualist ensures its own survival by rendering sperms incompetent to fertilize eggs unless they also contain the mutualist. Uninfected females do not lay fertile eggs after mating with infected males.

Biochemical investigations of the mosquito-filaria relationship were undertaken by Jaffe under Grant AI-08261 in order to detect changes which may occur in Aedes aegypti when they become infected with Brugia pahangi. The general properties of three mosquito enzymes have been characterized and compared with the analogous enzymes from filariae. The ultimate aim is to develop chemical agents which would render mosquitoes unsuitable hosts, interrupting the transmission cycle. This should prove to be a fruitful new line of research.

The expense and effort involved in maintaining filarial parasites by conventional methods underscored the need for the development of standard procedures for cryopreservation of microfilariae and infective larvae of filariae. Efficient cryopreservation techniques would also allow for the shipment of human filariae from endemic to nonendemic areas. For these reasons, we initiated two contracts to develop and standardize methodology for the cryopreservation of sheathed and unsheathed microfilariae and infective larvae (Delta Primate Center, AI-62503 and University of Georgia, AI-62504).

E. TUBERCULOSIS

Research in the Tuberculosis Program is directed towards the isolation of more effective diagnostic antigens and the development of better anti-tuberculosis vaccines and vaccination methods.

Under Contract AI-22521, Dr. Robert C. Good is continuing a study in monkeys to determine the duration of protection of BCG vaccine administered by two different routes, i.e., inhalation and intracutaneous injection (IC). Previously, he demonstrated that inhalation of the aerosolized vaccine, as well as intravenous administration, conferred a higher degree of resistance to challenge than did intracutaneous administration. The protection afforded by aerosolized vaccine was found to be related to the total dose of viable units (vu) administered through the highest dose tested, 10^5 vu. In the present study with animals challenged eight weeks post vaccination, 67% of control (unvaccinated) animals and 23% of aerosol vaccinated and 34% of IC vaccinated animals were dead by the 24th week. By the 48th week, 56% of animals that received the aerosol vaccine, 67% of animals receiving the intracutaneous vaccine, and 92% of control animals were dead. The second challenge group was exposed to an aerosol of virulent tubercle bacilli 29 weeks post vaccination. By the 24th week, 17% of aerosol vaccinated, 25% of intracutaneous vaccinated and 50% of control animals were dead. At 54 weeks post vaccination, 67% of aerosol vaccinated, 50% of intracutaneously vaccinated and 84% of control animals were dead. The third group of animals was challenged 72 weeks post vaccination. By the 13th week post-challenge, none of the animals have died in either of the vaccine groups; however, one animal, or 8%, has died in the control group. On the completion of this third experiment, staff will evaluate the total experiment to determine future plans. As an adjunct to this study, 32 monkeys are being used to determine the effects of aerosol vaccination in previously sensitized animals. The monkeys have been sensitized and are now scheduled for post-sensitization exposure to BCG. The baseline pulmonary function tests have been performed on all animals, and these tests will be repeated according to protocol after re-exposure.

In another tuberculosis vaccine study (AI-42555) which Dr. Good has concluded, a mycobacterial ribosomal preparation of M. tuberculosis, Strain H37Ra, was shown to be active in conferring protective immunity in guinea pigs. The challenge with the highly virulent Erdman Strain of M. tuberculosis presented a severe test of the immunizing activity. Whether challenged by aerosol or by intraperitoneal injection, activity of the vaccine was found to be dose related, but was not equivalent to protection conveyed by viable BCG cells. The immune response elicited by the ribosomal preparation suggests identification of the active immunogen present in this preparation. After thorough review and evaluation of the results of this study, a limited trial may be warranted. In addition to the vaccine contract research, many related aspects of tuberculosis pathogenesis and immunity were clarified through various grant activities of the Program. Dr. Donald W. Smith (R22 AI-07485-08A2) developed a guinea pig model which permits examination of the bacillemic phase of tuberculous infection. By the use of this model, he has examined the influence of BCG vaccination on the tuberculous infection and found that the hematogenous seeding of the lungs and the extent of the bacillemia in BCG vaccinated animals was largely controlled.

His findings have prompted the tuberculosis unit of the World Health Organization to consider the adoption of the "Magnitude of Bacillemia" animal model for the purpose of comparing the potency of the principal seed lots of vaccine throughout the world.

Dr. Frank M. Collins, (R22 AI-09962-03) quantitated the effect of calf thymosin on the ability of T-cell depleted mice to express an effective anti-tuberculous immunity. The BCG infected T-cell depleted mouse constituted a valuable biological assay for thymosin activity in vivo, but this test was found to be very cumbersome and time consuming.

Dr. Arthur M. Dannenberg, Jr. (Grant R22 AI-08876) continued to clarify the role of activated macrophages in the pathogenesis of tuberculosis. Using plastic skin chambers, the in vivo secretion of macrophage enzymes over dermal BCG lesions were studied. The highest concentration of the enzymes were found at the time tuberculin hypersensitivity had developed and tuberculin-like products of the bacilli were still present. It appears that enzyme secretion, regurgitation and release by cell death are all increased when the macrophages in the BCG lesions become highly activated.

Dr. Samuel B. Salvin (Grant R22 AI-08528-08) has shown that tuberculin sensitive mice that release soluble mediators of delayed hypersensitivity (lymphokines) into the circulation after stimulation with partially purified antigens from tubercle bacilli show a marked resistance to aerosol challenge with a virulent strain of M. tuberculosis (H37Rv). From studies undertaken to identify the source of the in vivo produced lymphokines, both B- and T-cells are implicated, not singly, but in a complex interaction which is being investigated further. In addition, the investigator has shown that the sera containing the lymphokines exhibit active tumor suppressive properties. It appears that these lymphokines are not species-specific, and could therefore be prepared in one species and used therapeutically in others.

Dr. John David (Grant R22 AI-10921-05) continues to investigate the mechanism of cellular immunity and hypersensitivity. Special techniques were undertaken to identify the lymphocyte subtypes producing migration inhibitory factor (MIF) and leukocyte inhibitory factor (LIF). The studies were initiated in the murine system, a system in which the present advances in genetics and cell surface antigens makes it possible to determine what subtype of T-cell is involved in mediator production and the relationship of these mediator to the product of immune response genes.

The contract effort to fractionate, isolate and characterize antigens from unheated M. tuberculosis continues. An unheated filtrate of M. tuberculosis (H37Rv strain) was subjected to preparative gradient acrylamide gel electrophoresis and 26 fractions were isolated by Dr. George L. Wright, Jr. (AI-32503). Dr. Thomas M. Daniel (AI-42525) using immunoelectrophoretic and immunodiffusion analyses identified with certainty, mycobacterial antigens #1, 2, 5, 6, and 7 of the U.S.-Japan Reference System. Dr. Robert C. Good (AI-42522) found that fractions 9 through 15 were significantly reactive intradermally in homologously sensitized guinea pigs and exhibited species specificity. Dr. Robert A. Goldstein (AI-52508) found that fractions 8 through 18 induced significant blasto-

genic responses in lymphocytes from tuberculin positive healthy subjects and patients with atypical mycobacterial infections, although species-specific reactivity was not obvious. In both biological test systems it appeared that antigens #5 and 6 are the reactive antigens. Because of these results, efforts are underway to prepare homogeneous samples of these antigens for further testing (Dr. Alva H. Johnson, new Principal Investigator, AI-32503).

In addition, Dr. Wright recently prepared 18 fractions from cell extracts of M. tuberculosis. The in vivo and in vitro testing procedures are underway. The results from these experiments will determine whether or not cell extract antigens should be purified further. Complementary grant research was conducted by several investigators interested in mycobacterial antigens. Dr. Lewis F. Affronti (Grant R22 AI-08529) continued to study the PPD fractions of seven typical and atypical mycobacterial strains. The protein concentration and skin test reactivity were determined. PPD-Gause 7% fraction exhibited species specificity and this fraction was analyzed for amino acid content.

Dr. Mayer B. Goren (Grant R22 AI-08401-08) continues to study the surface components (principally lipids) of mycobacteria. Currently, he has isolated a trehalose mycolate (a cord factor-like substance) from harvests of intracellular M. lepraemurium. This constitutes the first identification of such a glycolipid from in vivo grown bacilli. In synthesis studies an analog of trehalose and protein conjugates of trehalose derivatives were prepared for immunological assessments in animals.

Strains of mycobacteria from an authenticated, highly characterized, and taxonomically classified culture collection (AI-52529) continue to be supplied to qualified scientists in tuberculosis research and related fields. On this contract two new strains, M. microti and M. simiae, were accessioned this year by Dr. Maurice Lefford. This brings the total number of accessioned strains available for distribution to 160. At the present, seven recently acquired strains are being screened with a view to possible accession.

Dr. J. Kenneth McClatchy (AI-02079) is continuing the serological classification of mycobacteria other than M. tuberculosis obtained from infections in man. In the past year more than 800 strains were sent for serotyping from Australia, Canada, The Netherlands, West Germany, Sudan, United Kingdom, Denmark, Brazil, and the United States. In addition, antisera for performance of the typing procedure were sent to approximately 20 investigators in the U.S. and abroad. A microbiologist from Brazil visited in the laboratory for ten days to learn the technique. To date, 23 cultures of M. szulgai, a newly recognized pathogen in man, have been received. A definitive study is underway to characterize and classify this important organism. Antisera are being developed for the serological identification of rapid growing organisms of the M. chelonei complex. These organisms (subspecies abscessus and subspecies borstilense) have recently been isolated from mechanical heart valves implanted in human patients.

A study of the serological and biochemical characteristics of a large group of unclassifiable organisms isolated from patients has also been initiated. These organisms appear to be an intermediate group between M. scrofulaceum and M. intracellulare. Since most of the strains are considered to be of clinical

significance, it is important to be able to recognize and identify these organisms with accuracy. [illegible] contracts (AI-52529 and AI-02079) [illegible] have been supported by granted projects that have resulted in 12 publications this year.

F. VIRAL DISEASES

Research in the Viral Diseases Program is limited to studies on rabies and on arboviruses with emphasis on dengue and the pathogenesis of hemorrhagic fever.

Rabies

One of the major emphases of Contract AI-12199 at the Michigan State Department of Public Health was on evaluation of the Rapid Fluorescent Focus Inhibition Test (RFFIT). The RFFIT was shown to be more reproducible than the mouse serum neutralization test. Data indicate that the usual small variation in time and temperature in the day to day operation of a laboratory would not produce an observable change in RFFIT results since the titer of the serum varied inversely with the challenge virus dose.

EDTA-extracted glycoprotein was shown (Contract AI-42554--University of Michigan) to result in an adequately purified preparation with antigenic activity. While data is fragmentary, the objective of binding EDTA extracts onto larger vaccine complexes is a viable concept for the production of a sub-unit rabies vaccine.

Grant-supported field investigations (Fischman--AI-12107) of rabies in Virginia led to the observation of a species-specific geographically confined occurrence of fox rabies in the U.S. over the past 30 years. These rabies areas correlate in a striking manner with U.S. Geological Survey maps of the caves and caverns in the country. This epidemiological lead is to be pursued. The same grantee is developing new diagnostic procedures using skin snips from the face, head and back of the neck of mice. Fluorescent antibody analysis of this material permits rabies diagnosis from three to five days in advance of signs.

Dengue

Grantees in Malaysia (Rudnick AI-11540) believe that they have discovered the forest canopy vector of dengue, the final link in the jungle dengue cycle. During the past year, an additional strain of dengue virus--type 4--was isolated from the flood of a sentinel monkey in the high canopy bait trap. All of the isolates have been recovered from sentinel monkeys in the forest canopy, while none has been recovered from monkeys held in the forest at ground level. The canopy-dwelling mosquito Aedes niveus will descend to the ground to feed on man, thus allowing transfer of the virus from the forest to the human environment. Attempts to correlate the jungle cycle in man are underway as are attempts to establish a laboratory colony of A. niveus.

In view of the potential role of immune complexes in the pathogenesis of the

dengue hemorrhagic shock syndrone, work has been directed at the measurement of immune complexes in biologic fluids (Zimmerman--AI-11751). Reduction of C1Q uptake by sensitized cells is a precise measure of the amount of immune complexes present in serum. It is now possible to quantitate immune complexes in terms of micrograms per milliliter. The test has been applied to several hundred sera from patients with dengue hemorrhagic fever. The evaluation of the sensitivity and specificity of the test is underway.

II. Research Resources - International Centers for Medical Research Program

In response to the expressed desire of the Congress (P.L. 86-610, a program for the International Centers for Medical Research was established in 1960 by the National Institutes of Health "to advance the status of the health sciences in the United States and thereby the health of the American people" by expanding collaborative research between U.S. universities and selected foreign institutions and investigators.

The four grants funded under this Program are awarded for five years with continued support contingent upon program review at the end of each year of operation. One of the most important features of this program is that of long-term support, as contrasted with the transiency which has characterized a number of other international projects. This is a necessary ingredient for the full development of university careers in science which will effectively utilize the international medical experience of individual scientists. A major advantage of this program is that it provides opportunities for established and highly competent investigators to exploit valuable research opportunities abroad without losing their university affiliations and faculty appointments. The long-term support of the ICMR's does not represent a form of foreign aid. Rather the primary objective is a collaborative effort in biomedical research of specific relevance to the health of the American people and to the U.S. scientific community. The four current ICMR grants were renewed for an additional five years in FY75.

Purpose and Scope

The ICMR Program has as its principal objective the provision of high quality research and scientific opportunities for Americans in the broad fields of geographic medicine, and in response to the special opportunities existing within each ICMR framework. The length of the research period overseas is a discretionary concern of the ICMR program director, and is obviously determined by both the interests of the prospective investigator and the specific opportunities available within the given ICMR unit. Consistently, the emphasis is upon the quality rather than the quantity or duration of the research programs.

The aggregate ICMR units are serving increasingly as a national resource for utilization by senior as well as less experienced scientists to create a comparatively modest pool of investigators with a sustained career interest in international or geographic biomedical research. In this regard, ICMR program directors are encouraged to establish a selective, interdisciplinary scientific program. Their activities encourage and accommodate a number of parent university departments other than the department which primarily sponsors the ICMR operation. Particular advantage is taken of special strengths within the overall parent university, including opportunities in health-related fields outside of the classical confines of tropical medicine.

During FY71, the NIH initiated a program element within the overall ICMR framework. Designated as the ICMR Research Associate Program (IRAP), this centrally coordinated activity is specifically designed to recruit investigators on a

national basis and specifically from universities that do not have an ICMR.

As far as is possible, the ICMR core grant is used for research support at the off-shore ICMR site rather than at the parent, domestic university. In this regard it is accepted that some preparation preliminary to an overseas assignment is frequently necessary or desirable, and as many items of equipment and supplies as feasible are purchased in the United States.

Program

The four institutions participating in the ICMR Program at present, and their respective areas of research interests are as follows:

1. The University of California ICMR under the direction of Dr. Albert Rudnick is based in San Francisco and in Kuala Lumpur, Malaysia. Current research interests include the epidemiology of the jungle cycle of dengue with related vector studies, the relationship of behavior to the epidemiology of infectious diseases, traditional Chinese medical practices, host-parasite interactions in relation to biocontrol of schistosomiasis and rural health care.

2. The Johns Hopkins University ICMR in Baltimore with its overseas site in Dacca, Bangladesh, is emphasizing research on diarrheal diseases including cholera, shigellosis, immunology and infection and the epidemiology of malaria. The Program Director is Dr. Frederik Bang.

3. The University of Maryland ICMR in Baltimore and Lahore, Pakistan is under the direction of Dr. Richard Baker. Research in Lahore is centered around an integrated program on the genetics and field ecology of mosquito vectors. Compound chromosome techniques have been developed and a pilot field program of biological control is about to be initiated.

4. The Tulane University in New Orleans has an overseas ICMR base in Cali, Colombia. Dr. Paul Beaver and Dr. Thomas Orihel are directing a broad research program including studies in several general sections--infectious diseases (trypanosomiasis, malaria, echinococcosis, filariasis, mycoses, cytomegalovirus diseases), immunology (influence of nutrition on immune response, cell mediated immunity in chronic infections), nutrition, and behavioral science.

PART A

Individual Contract and Agreement Tabulations

PROGRAM: Prevention, Treatment and Diagnosis of Infectious Diseases - U.S.-Japan Program

BRANCH: GMB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
CHOLERA	
Banwell, John G. AI 4-2549 U. of Kentucky $ -0-	Study of incidence of diarrhea caused by enterotoxin-producing E. coli. Epidemiological surveillance contract for obtaining the incidence and importance of enterotoxin in diarrheas in various populations. There is some indication of heat-stable toxin produced by E. coli being associated with diarrhea in the rural population being studied in Kentucky.
Craig, John P. AI 0-2091 Research Foundation State U. of New York $86,000	Operation of a laboratory for evaluation and measurement of cholera toxins, antitoxins, and toxoids. Maintains a laboratory and talent capable of performing antitoxin combining power (ATCP), mouse challenge studies and permeability factor (PF) measurements in rabbit skin. Essentially a service contract. The adjuvant effect on antitoxin titers found in rabbits was not demonstrable in human volunteers. Also, the interval between the two primary boosters had no effect on antitoxin titers in the volunteer studies. There is an unexplained difference in antitoxin response in U.S. volunteers and Bengali's, 30-80 units/ml and 212/ml, respectively.
Guerrant, Richard AI 4-2548 U. of Virginia $ -0-	Study of incidence of diarrhea caused by enterotoxin-producing E. coli. Epidemiological surveillance contract for obtaining the incidence and importance of enterotoxin in diarrheas in various populations. Studies are being done

	in Brazil on a population of children and adults, in the U.S. on an Indian reservation, and in a university health unit, and in a group of families. It is becoming apparent that enterotoxin-producing E. coli play a highly variable role in association with acute diarrheal illness.
Peterson, Johnny AI 0-2101 U. of Texas Med. Branch at Galveston $96,000	Study of cholera toxoids in human volunteers. Maintains a prison volunteer capability to test the ability of cholera immunogens to produce circulating antibody and/or antitoxins. Essentially a service contract. Dose response curves inoculating up to 400 mcg of cholera toxoid are being obtained. These higher doses did not cause any systemic effects in volunteers. The 2500 sera obtained are still in the process of being titered.
Pierce, Nathaniel F. AI 0-2051 Johns Hopkins U. $102,000 $10,000 (est.)	Evaluation of purified cholera toxins and toxoids as immunizing agents against cholera. Maintains a laboratory and talent for performing antitoxin titrations in ileal segments in rabbits and also provides the dog model for studying vibrio infections. Essentially a service contract. The challenge studies in dogs show a clear superiority of a combined immunizing regimen with cholera toxoid employing parenteral (SC) priming and oral boosting (consisting of 10 daily doses of 50 mcg toxoid). Evidence indicate this protection is due to local antitoxin production, since protection is present in the absence of detectable circulating antitoxin.
Smith, Harry L., Jr. AI 3-2502 Jefferson Medical College $38,000	Operation and maintenance of a cholera vibrio reference laboratory. Makes high quality cholera typing sera and distributes it at no cost to laboratories around the world as well as within the U.S. Maintains a vibric collection from all known outbreaks of cholera and non-cholera vibrio-associated diarrheas. Maintains a record of the characteristics of these cultures for technical and epidemiological comparisons. Essentially a service contract. The contractor received 137 vibrio cultures during the year, sent out 10 cultures and 22 vials of typing sera. The cooperation with CDC and Japanese counterparts to arrive at a common

vibro characterization and serotyping scheme is continuing. The number of recognized serotypes has been decreased from 100 to 79 so far, and progress is being made toward a unified system.

LEPROSY

Bloom, Barry
AI 6-2501
Yeshiva U.
$32,000

New contract to conduct in vitro tests to determine changes in cell-mediated immunity in leprosy patients receiving immunotherapy with Transfer Factor.

Guttman, Helene
AI 4-2556
U. of Illinois
$ -0-

Studies on the cultivation of M. lepraemurium. Fetal calf serum appears to be a better supplement to Nakamura's basal medium than goat serum. This project phased out March, 1976.

Kirchheimer, Waldemar
1-Y01-AI-60001
U.S.P.H.S. Hospital
Carville, Louisiana
$96,000

Establish and maintenance of an armadillo colony infected with M. leprae.

Levy, Louis
2-Y01-AI-10004
U.S.P.H.S. Hospital
San Francisco
$24,000

Microbiological studies on leprosy skin biopsy specimens and studies on antileprotic drugs to evaluate activity against M. leprae in the mouse foot pad model.

Peters, John
AI 0-2283
Stanford Research Institute
$18,000 (est.)

Study the metabolism of rifampin (RFM) in leprosy patients receiving this drug or dapsone for therapy. Techniques developed for accurately measuring 0.1 mg/ml in plasma. Dapsone and other sulfones do not interfere with this technique.

Reich, Claude
AI 5-2509
Leonard Wood Memorial
$149,000

Conduct clinical trials of candidate chemotherapeutic compounds in leprosy patients in Cebu, Philippians. Long term response to various drug regimens - rifampin plus dapsone and rifampin plus DADDS are evaluated in lepromatous patients. Rifampin continues to be bactericidal and reduces the BI (bacterial index) more rapidly than DDS or DADDS.

Shepard, Charles
2-Y01-AI-90905
Center for Disease Control
$90,000

Continue to investigate the activity of dapsone and other antileprotic drugs using a pharmacokinetic method in the mouse foot pad model. Drug combinations are currently being evaluated for DDS-resistant infections.

Spitler, Lynn
AI 5-2500
U. of California
$ -0-

Preparation of transfer factor (TF) from Dharmandra antigen positive and negative normal volunteers; studies on ability to passively transfer in vivo and in vitro correlates of hypersensitivity are in progress. Anticipated project will be completed by August, 1976.

Worth, Robert
AI 5-2507
U. of Hawaii
$8,000

Collection and analysis of data on leprosy in the Pingelapese population. This study will not be completed until 1980. It is anticipated the prophylactic effect of DADDS therapy and the required threshold dose of this drug to interrupt the transmission of leprosy will be ascertained.

MALNUTRITION

Coursin, David
HD 4-2816
St. Joseph Hospital
$ -0-

Instrumentation design for electrophysiological studies. The portable electronic acquisition system is now in the operational prototype stage, capable of producing programmed pattern stimuli and recording two channels of electrophysiological data along with three event monitors.

Juliano, B.
AM 7-0726
International Rice Research Institute
$9,000 (est.)

Field studies to evaluate rice with high protein content in Filipino children - demonstration project for nutritional supplementation.

Katz, Michael
HD 4-2859
Pan American Health Organization
$55,000 (est.)

Continuation of studies on the interaction between malnutrition and infection.

Neumann, Charlotte
HD 5-2851
U. of California
Los Angeles
$ -0-

Conduct studies on the effects and interrelationships of intrauterine malnutrition and the developing immune system in the new born.

PART A - GMB - Continued

PARASITIC DISEASES

Bayne, Christopher
AI 2-2527
Oregon State U.
$ -0-

To develop primary cell cultures and to establish permanent cell lines derived from tissues of fresh molluscs ...
A simple method for freeze preservation of Biomphalaria glabrata cells was developed by using 10% DMSO in insulated ampules transferred from room temperature to minus 16°C, to minus 86°C, to minus 196°C at approximately one hour intervals.

Blankespoor, Harvey
AI 4-2504
U. of Michigan
$132,000

To supply investigators with mammals and snails infected with various strains of Schistosoma mansoni, S. japonicum and S. hematobium ...
Two hundred and twenty two requests were filled for 33 investigators. Nine thousand two hundred infected mammals and 1800 infected snails were shipped.

Lowrie, Robert
AI 6-2504
Delta Primate Center
$24,000

To develop and standardize methodology for the cryopreservation of sheathed and unsheathed microfilarial and infective larval of filariae of public health importance ...
This contract becomes effective 1 June 1976.

McCall, John
AI 5-2519
U. of Georgia
$75,000

To supply investigators with animals and vectors infected with various filarial species ...
One hundred and thirty requests for filariasis research materials were filled for 28 investigators representing 24 institutions.

McCall, John
AI 6-2503
U. of Georgia
$19,000.

To develop and standardize methodology for the cryopreservation of sheathed and unsheathed microfilarial and infective larval of filariae of public health importance ...
This contract becomes effective 1 June 1976.

TUBERCULOSIS

Daniel, Thomas
AI 4-2525
Case Western Reserve U.
$ -0-

To test serological mycobacterial products. Fractions of cell extracts (CE) and culture filtrates of 13 mycobacterial species were analyzed by microimmunodiffusion and immunoelectrophoresis. The CE fractions contained relatively less antigenic material than the similarly prepared culture filtrate fractions analyzed previously. Antigens CE 1 and CE 2

of the U.S.-Japan reference system were positively identified. The study of the culture filtrates showed that antigens 1 and 2 are widely distributed among mycobacteria. Antigen 5 was found only in M. kansasii and M. xenopi. Antigen 6 was found in these plus M. szulgai. Antigen 7 was found in M. fortuitum, M. smegmatis and M. phlei.

Goldstein, Robert
AI 5-2528
Medical Research Associates, Inc.
$ -0-

To test mycobacterial fractions of H37Rv culture filtrate and cell extract by lymphocyte blastogenesis. The bioactivity and specificity of 23 fractions of M. tuberculosis cell extract were determined. Variations in duration of lymphocyte culture, variations in antigen concentration, and the influence of autologous and homologous plasma were tested. To date, using the standard 96 hour harvest time and antigen concentrations previously tested, significant bioactivity was found in fractions #7 through #12 with the peak activity appearing in fraction #9. These results were obtained in patients who had culture proven infections with M. tuberculosis as well as in selected individuals with atypical mycobacterial infections. Persons with other granulomatous pulmonary infections, failed to respond with the notable exception of one person with aspergillosis, in whom bioactivity was noted in the earlier fractions.

Good, Robert
AI 2-2521
Hazleton Laboratories America, Inc.
$32,000

To study the duration of protection of BCG aerosol vaccination in monkeys. Aerosol BCG vaccination has proved highly effective in the monkey model and has been shown to be dose dependent. In the animals challenged 8 weeks postvaccination, a significant difference was found between the vaccinated and nonvaccinated groups. By the 48th week after challenge, 56% of the aerosol vaccinated, 67% of the intracutaneously vaccinated and 92% of the nonvaccinated had died . In the animals challenged 29 weeks postvaccination, by the 54 week after challenge, 67% of the aerosol vaccinated, 50% of the intracutaneously vaccinated, and 84% of the nonvaccinated had died. A third group of animals have been challenged 72 weeks postvaccination and data pertaining to their survival will be forthcoming in FY 77.

Good, Robert
AI 4-2522
Hazleton Laboratories America, Inc.
$49,000

To test the skin reactivity of mycobacterial fractions in guinea pigs. Of the 26 culture filtrate fractions tested, fractions 9 through 15 were significantly reactive intradermally in homologously sensitized guinea pigs and exhibited species specificity. Bioequivalent doses have been determined for 18 cell extract fractions in homologously sensitized animals. Subsequent testing in heterologously sensitized animals will soon be completed.

Good, Robert
AI 4-2555
Hazleton Laboratories American, Inc.
$ -0-

Evaluation of Youmans' ribosomal vaccine in guinea pigs. The data indicates a protective effect which is dose dependent. The final report is being prepared.

Lefford, Maurice
AI 5-2529
Trudeau Institute
$ -0-

Authentication, maintenance, and distribution of a mycobacterial culture collection. To supply investigators throughout the world with cultures of biochemically characterized and virulence tested mycobacteria. During the past year, 901 cultures, 190 vials of PPD, 23 antigen-antibody kits and 16 vials of other serological reagents were distributed. The collection now consists of 160 accessioned strains. A study was made of three media for the recovery of preserved cultures. There was essentially no difference between 7H-9 broth, 7H-10 agar and ATS medium.

McClatchy, J. K.
AI 0-2079
National Jewish Hospital
$52,000

To serve as a reference laboratory for the identification of unknown strains of mycobacteria and to provide standard sets of antisera suitable for serotyping certain groups of mycobacteria together with the corresponding reference strains. More than 800 strains were sent for serotyping from Australia, Canada, the Netherlands, West Germany, Sudan, United Kingdom, Denmark, Brazil and the U.S. Antisera for performance of the serotyping procedure was sent to 20 investigators in the U.S. and abroad. Antisera for the serological identification of the rapid grower M. chelonei complex is being developed. This organism has been isolated from mechanical heart valves in humans.

Youmans, Guy
AI 5-2501
Northwestern U.
$ -0-

To prepare a mycobacterial ribosomal vaccine and monitor the inoculation of it in guinea pigs. The vaccine was prepared, tested in mice prior and post inoculation of the guinea pigs. The vaccine was judged to be potent. The results of the guinea pig experiment (contract AI 4-2555) are being prepared for publication.

Wright, George Jr.
AI 4-2503
Eastern Virginia
Medical School
$47,000

To fractionate and characterize mycobacterial antigens by gradient acrylamide gel electrophoresis (GAGE). Culture filtrate fractions 9 through 15 have been selected on the basis of bioactivity and specificity for subfractionation. Fractions from cell extract have been prepared and are now being tested by other contractors.

VIRAL DISEASES

Mitchell, John
AI 1-2199
Michigan Department
of Public Health
$56,000 (est.)

To determine the post-exposure efficacy of various inactivated rabies vaccines in guinea pigs ...
The Rapid Fluorescent Focus Inhibition Test (RFFIT) was evaluated with respect to reproducibility, incubation and temperature for neutralization and effect of challenge virus dose ... Adsorption of rabies antigen to Al-P04 in post-exposure protection was not particularly effective. Rabies virus preparation from various infected tissues from diagnostic labs were evaluated as possible challenge virus. It is determined that none of this material would be suitable for challenge experiments.

PART B

PUBLICATIONS

I. Contractor Publications

CHOLERA

1. Guerrant, R.L., Moore, R.A., and Sande, M.A.: Toxigenic E. coli in infantile diarrhea in Brazil. New Engl. J. Med. 293:567-573, 1975.

2. Korzeniowski, O.M., Moore, R.A., Titericz, E. and Guerrant, R.L.: Diarrea com Desidratacao nas Criancas Examinadas no Hospital Infantil en Florianopolis SC: Etiologias Potencias e Implacacaoes para Terapie. Revista Medica da Associacao Catarienense de Medicina, 1976.

3. Pierce, N.F. and Reynolds, H.Y. Immunity to experimental cholera. II. Secretory and humoral antitoxin response to local and systemic toxoid administration. J. Inf. Dis. 131:383-389, 1975.

LEPROSY

1. Binford, C.H., Guinto, R.S., Levy, L., Shepard, C.C., and Tolentino, J.G. Rifampin therapy of lepromatous leprosy. Am. J. Trop. Med. Hyg. 24:475-484, 1975.

2. Levy, L. and Peters, J.H. Susceptibility of Mycobacterium leprae to dapsone as a determinant of patient response to acedapsone. Antimic. Agents Chem. 9:102-112, 1976.

3. Murray, J.F., Jr., Gordon, G.R., and Peters, J.H. Determination of rifampin and desacetylrifampin in plasma. Pharmacologist 17:266, 1975.

4. Murray, J.F., Jr., Gordon, G.R., and Peters, J.H. The influence of rifampin, desacetylrifampin, B663, and thalidomide on the determination of dapsone and monoacetyldapsone by a chromatographic-fluorometric method. Lep. Sc. Mem. 1976.

5. Peters, J.H., Gordon, G.R., and Karat, A.B.A. Polymorphic acetylation of the actibacterials, sulfamethazine and dapsone, in South Indian subjects. Am. J. Trop. Med. Hyg. 24:641-648, 1975.

6. Peters, J.H., Murray, J.F., Jr., Gordon, G.R., and Jacobson, R.R. The disposition of sulfoxone and sulasulfone in leprosy patients. Lepr. Rev. 46:171-180, 1975.

7. Peters, J.H., Shepard, C.C., Gordon, G.R., Rojas V.A., and Elizondo S.D., The incidence of DDS resistance in lepromatous patients in Costa Rica: Their metabolic disposition of DDS. Tenth Joint Conference on Leprosy, U.S.-Japan CMSP, NIH, Bethesda, Maryland, 164-173, 1975.

PART B GMB/LEPROSY - Continued

8. Russell, D.A., Worth, R.M., Jano, B., Fasal, P., and Shepard, C.C. Prevention of leprosy by acedapsone. Lancet 771, 1975.

MALNUTRITION

1. Eggum, B.O. and Juliano, B.O. Higher protein content from nitrogen fertiliser application and nutritive value of milled-rice protein. J. Sci. Food Agr. 26:437-439, 1975.

2. Juliano, B.O. and Beachell, H.M. Status of rice protein improvement. IN: CIMMYT/Purdue Univ., High-Quality Protein Maize, Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pennsylvania, 457-469, 1975.

3. Roxas, B.V., Intengan, C.L., and Juliano, B.O. Effect of protein content of milled rice on nitrogen retention of Filipino children fed a rice-fish diet. Nutr. Rept. Intern. 11:393-398, 1975.

4. Villareal, R.M. and Juliano, B.O. Some properties of rice leaf protease. Kimika Bull. Chem. Soc. Philippines. 20-25, 1976.

PARASITIC DISEASES

1. Bayne, C.J., Owczavazk, A. and Noonan, W. In vitro cultivation of cells and a microsporidian parasite of Biomphalaria glabrata. Ann. N.Y. Acad. Sci. 266:513-527, 1975.

2. Blankespoor, Harvey and Henry Van der Schalie. Attachment and penetration of miacidia observed by scanning electron microscopy. Science. 191:291-293, 1976.

3. Hosaka, Yukio and Elmer Berry. Schistosome miracidial immobilization caused by tissue - extracts prepared from various species or strains of snails. Japanese J. of Parasitology. 24:318-331, 1975.

4. Klei, Thomas. Intracellular rickettsia-like organisms in developing Dipetalonema viteae. J. Parasitology - In Press.

5. Klemm, Donald. Leeches found in North American mollusks. Malacological Review. 9:63-76, 1976.

6. Liang, Yung-san and Henry van der Schalie. Cultivating Fossaria bulimoides a snail host of the liver fluke, Fasciola hepatica. Malacological Review 8:123, 1975.

7. Liang, Yung-san and Henry van der Schalie. Cultivating Lithoglyphosis aperta, a new snail host for Schistosoma japonicum, Mekong strain. J. of Parasitology 61:915-919, 1975.

PART B GMB/PARASITIC DISEASES - Continued

8. McCall, John. A simple method for collecting infective larval of Litomosoides carinii. J. Parasitol. In press.

9. Sturm, P., Cory, M., Henry, D., Zeigler, J. and McCall, J. Antifilarial agents. 3 - aminopyrrolidine and 1, 4 Diazabicyclo (3.2.1) octave derivatives as analogs of diethylcarbamazine. J. Medicinal Chemistry. In press.

In addition, as stated in the narrative, many of the Institute's grantees are dependent upon both the Schistosomiasis and Filariasis supply contracts for animals and vectors infected with various strains. Grantees who utilize these contracts published 54 papers during the past year. These are not cited here since grant management is the responsibility of Extramural Programs.

TUBERCULOSIS

1. Daniel, T.M., Good, R.C., and Janicki, B.W.: Immunoelectrophoresis of Mycobacterium tuberculosis antigens. Comparative analysis of cell extract and culture filtrate antigens. Amer. Rev. of Respir. Dis. 112:639-644. 1975.

2. Janicki, B.W., Wright, G.L., Jr., Good, R.C., and Chaparas, S.D.: Comparison of Antigens in Sonic and Pressure Cell Extracts of Mycobacterium tuberculosis. Infect. Immun. 13:425-437, 1976.

II. Staff Publications

1. Lambert, P.D., and Martin, Angela: What You Should Know About Tuberculosis. Pharmacy Times. 42:50-56, 1976.

2. Miller, C.E. and Feeley, J.C. Competitive effects of intestinal microflora on Vibrio cholerae in gnotobiotic mice. Lab. Animal Sci. 25 (4):454-458, 1975.

PART C

Research Conferences and Workshops

In addition to program elements involving research grants and contracts, the U.S.-Japan Cooperative Medical Science Program includes a provision for regular scientific conferences and workshops. These include joint meetings with Japanese counterpart scientists as well as special conferences for American investigators working in the scientific areas of special interest to a given Panel. These workshops and conferences serve to stimulate interest and enthusiasm which leads to the development of new scientific approaches to the seven disease categories within the Program, and they also facilitate the timely exchange of research data, methods and protocols. The Joint U.S. and Japanese Panels meet annually, alternating between locations in the United States and Japan. A notation of conferences and workshops sponsored during FY 1976 follows:

Meeting Title and Location	Sponsoring Panel

July 11, 1975
U.S. Delegation Meeting
State Department
Washington, D.C.

Members of the U.S. Delegation met at the State Department along with Staff and Panel Chairmen. The Delegation listened to reports of progress in the seven disease categories presented by the Panel Chairmen or their designate. Plans were made for the Joint meetings in October.

July 21-24, 1975 — Environmental
Joint Environmental Conference
Seattle, WA

The conference was concerned with *in vitro* Mutagenicity and Carcinogenicity Tests. The topics covered were: Special topics in mutagenesis and carcinogenesis, qualitative correlation between carcinogenicity and mutagenicity, general criteria used to determine mutagenic potential in experimental organisms, current status of testing program on mutagenicity of chemical carcinogens, correlation between carcinogenicity and unscheduled DNA synthesis and correlation between carcinogenic activity and *in vitro* transformation.

September 8-12, 1975 — Tuberculosis
Joint Tuberculosis Conference
Bozeman, MT

The conference was concerned with the chemistry and biochemistry of mycobacterial constituents, immunity in tuberculosis, tumor regression mediated by BCG or mycobacterial components, and delayed hypersensitivity. Other related subjects were included on the agenda.

Meeting Title and Location (con't)	Sponsoring Panel
September 27-29, 1975 Joint Leprosy Conference Bethesda, MD	Leprosy

This was the Tenth Joint Conference on Leprosy research and included presentation of both U.S. and Japanese investigations.

October 14-16, 1975 Joint Viral Diseases Conference San Antonio, TX	Viral Diseases

Formal presentations and round-table discussions emphasized the molecular biology, pathogenesis and immunology of rabies and the epidemiology, molecular biology, cell culture and immunogenicity of dengue and other arboviruses.

October 26, 1975 Leprosy Chemotherapy Workshop Bethesda, MD	Leprosy

Prior to the Joint Leprosy Conference, a one-day workshop was held to discuss the state of the art of leprosy chemotherapy and to determine what future drugs and drug regimens should be pursued.

October 27-29, 1975 Joint Parasitic Diseases Conference Bethesda, MD	Parasitic Diseases

Included among the papers presented at the Tenth Joint Conference on Parasitic Diseases were those on the epidemiology, vector biology, biochemistry and pharmacology and immunology of both schistosomiasis and filariasis. In addition, two minisymposia directed attention to "Reproductive and neural physiology of Schistosomes" and "Animal models in filariasis." Schistosomiasis in China was discussed at an evening session by a Panel of U.S. Parasitologists who had recently visited the Peoples Republic of China under the auspices of the National Academy of Sciences.

October 29, 1975
Joint Subcommittee on Program Review and Planning Meeting
National Institutes of Health
Bethesda, MD

The Joint Subcommittee on Program Review and Planning met the day before the Joint Committee meeting to discuss agenda items and the publishing of the Second Five Year Report.

Meeting Title and Location (con't) | Sponsoring Panel

October 30-31, 1975
Joint Committee Meeting
National Institutes of Health
Bethesda, MD

The Joint Committee, composed of the U.S. Delegation and the Japan Delegation, heard and discussed the scientific reports of the U.S. and Japanese Panel Chairman. Publication details of the Second Five Year Report were discussed. The Joint Committee recommended that the review of the Leprosy and Tuberculosis Panels be initiated in 1976.

October 31, 1975 — Cholera
Dialogue on the Research Potential
of the Cholera Research Laboratory
Bethesda, MD

This meeting presented the unique characteristics of a well-equipped laboratory, two functional hospitals, and a large study population available in a developing country in Asia.

November 4-6, 1975 — Cholera
Joint Cholera Conference
New Orleans, LA

This meeting included sessions on *V. cholerae* and *E. coli* diarrheas; bacterial genetics; bacteriology and toxinology; enterotoxins; cholera vaccines and immunology.

December 3-5, 1975 — Malnutrition
Joint Malnutrition Conference
Berkeley, CA

This research conference was devoted to the biological and cultural sources of variability in human nutrition.

December 15, 1975 — Malnutrition
Workshop on Iron Metabolism
Saint Luke's Hospital
Denver, CO

This workshop was convened to bring together a group of experts to explore the present state of the art concerning the role of iron deficiency as a significant contributor to the problem of nutritional anemia in Southeast Asia and its impact on health in this area of the World. The participants of the workshop prepared a summary document including a list of recommendations for future projects.

Meeting Title and Location (cont'd) Sponsoring Panel

January 29-30, 1976
Joint Subcommittee on Program Review and
Planning Meeting
Honolulu, HI

Representatives from the U.S. and Japanese delegations met to plan for the Twelfth Joint Committee in September, 1976, in Tokyo, Japan. The Report of the Binational Review Panel was discussed and a Symposium planned just prior to the aforementioned meeting.

RESEARCH RESOURCES BRANCH

The need for well characterized reference reagents as an adjunct to research is well recognized and, in support of this concept, the Research Resources Branch (RRB) conducts a program which distributes a wide range of reagents to research scientists. During the last quarter of FY76 the reagents program of the Transplantation and Immunology Branch was merged with the ongoing programs of the RRB which already consisted of a wide range of seed viruses, mycoplasmas, allergens and corresponding antisera. In all program areas the Branch identifies reagents for which a critical need exists, has these produced or acquired through the contract mechanism, has them fully characterized by certification or quality control laboratories, and distributes them as reference reagents along with information and technical advice on reagent characteristics and use. During FY76 a new program was developed and initiated for the construction of safer phage and plasmid vectors for E. coli hosts that will facilitate research using DNA recombinant molecule technology.

I. Immune System and Disease

A. Asthma and Allergic Diseases Program - Allergen Reagents

A contract with the Harbor General Hospital resulted in the delivery during FY76 of an antihuman IgE serum produced in rabbits against the myeloma protein PS. It is anticipated that this reagent will be most useful in the Radioallergosorbent Test (RAST) which is now in widespread use for both research and diagnosis of allergic diseases. This reagent should be particularly useful to investigators in the NIAID Asthma and Allergic Disease Centers.

Delivery has been made of 500 mg of the Asc-1 allergen from the helminth Ascaris suum; this contract was initiated late in FY74 with the University of Western Ontario. This material has the facility of inducing high levels of IgE in laboratory animals and furthermore haptens can be easily coupled to it. This reagent will be useful to a variety of laboratories that are studying the synthesis and biological activities of IgE.

The Worthington Biochemical Corporation's contract to extract and purify Group Antigens I, II, and III from 9 kilograms of ryegrass (Lolium perenne) pollen has proceeded on schedule. Pilot runs allowed the contractor to adjust the technology and resulted in the antigens being produced according to the contract specifications. The bulk production process is completed and contractor testing has revealed that the three antigens have been produced in pure form at yields greater than expected based on the pilot-run data. Certification is in progress and subsequently delivery will be accepted near the end of FY76.

The Allergen Reagents Program acquired whole lyophilized venoms from honey bees, paper wasps, yellow jackets, yellow hornets and white faced hornets during FY76. These materials are being solubilized, processed, packaged and tested prior to application for an IND. These venoms will be employed in a collaborative study among four Asthma and Allergic Disease Centers and the

Walter Reed Army Medical Center. The study will compare the efficacy of whole venoms and whole beebody extracts in the diagnosis of the specific allergies by skin testing.

The Branch has acquired large volumes of human sera with high specific levels of IgE antibody directed against honey bee venom, whole ragweed pollen and Alternaria allergens. These sera will be of great assistance, along with other reagents in this program, for the internal standardization of in vitro tests and research procedures especially the RAST test.

The certification laboratory for the allergen reagent program remains at the Mayo Clinic. This central independent facility allows the branch to quickly verify the correctness of products produced by other contractors. In addition, this laboratory can expand the data base for the products beyond that available form producing contractors especially data regarding the biological and in vivo allergenic activities.

B. Tissue Transplantation- Reagents and Techniques

Since its inception in 1965 the program has fostered considerable research in defining the HLA region through the support of newer technology for histocompatibility testing and in making quality reagents available to worldwide research laboratories. During FY 76 contractors were funded to compare and develop new and various tissue typing techniques; produce a continuing supply of high quality histocompatibility typing sera; maintain and utilize lymphocyte reference panels to serve as a standard in screening sera and quality control testing; conduct research directed at discovering new antigen specificities or loci in the HLA region; sustain a serum repository; prepare the NIAID Tissue Typing Trays; and process, package and freeze dry bulk amounts of reagents in small aliquots for distribution.

Refinements in typing techniques have been developed and are under continued evaluation. An automated procedure using the Fluorochromasia Technique was developed by the University of Leiden contractor and the equipment or a modification of the prototype is now being used in other typing laboratories. Eight Mixed Lymphocyte Culture determinants were recognized with typing cells and research is continuing to define MLC and other B cell antibodies. Evidence was found to indicate that a new locus is adjacent to the MLC or D locus and separate from the B locus and it may be the human equivalent of the mouse Ia. Data were presented to support the splitting of antigens BW15, BW16, BW21 and B12 and illustrates the continuing need for the acquisition of sera with refined specificities.

The NIAID Serum Bank is managed by a contract with Flow Laboratories and currently contains over 1,700 sera with a total volume of 285 liters. These reagents are described in a Catalog of Tissue Typing Antisera which is periodically updated with user data on established antisera and new additional sera that have been acquired. During FY 76 a supplement of 300 pages was prepared and distributed to more than 400 laboratories. The availability of the reagents and the scope of the transplantation programs were publicized by exhibits at the American Association of Blood Banks, the National Kidney Foundation and the American Association of Clinical Histocompatibility Tesitng.

Additions to the Bank in FY 76 include 46 sera obtained by contracts with the Cleveland Clinic and the University of Leiden, 44 donated by 18 tissue typing laboratories and 74 purchased from 18 other sources. Eight typing laboratories performed quality control studies on the sera prior to their acquisition, thereby assuring reliability of the reagents. The demands on the Bank's resources are growing due to the increases in the number of world-wide solid organ and bone marrow transplants, increased studies on the relationship between HLA antigens and a variety of diseases; and donor-recipient matching for platelet and white blood cell transfusions. The Bank made 1554 shipments to 371 investigators during FY 76 and these were processed by the contract at Flow Laboratories.

Microbiological Associates Inc. continues as a sole contractor to produce and package for delivery to clinical transplant centers HLA serum typing trays. This is an attempt to standardize the reagents and techniques clinically employed in the United States for determining donor and recipient HLA antigenic profiles which are used in selecting the best solid organ or bone marrow graft for the recipient undergoing transplantation.

The current 72-well serum typing tray contains 69 well-studied reliable antisera which define 38 antigenic specificities of the A, B and C HLA loci. Every effort is made to keep the serum composition of these standardized NIAID trays constant over the years, and the quality of their production and effectiveness is regularly monitored by independent laboratories. The contractor has consistently produced high quality trays which are used in the transplant programs throughout the United States. During the FY 76 contract period, this contractor has produced approximately 34,000 trays.

A procedure was developed by the American Foundation for Biological Research to produce trays containing human lymphocytes with well characterized HLA antigens. However, it was discovered that these trays required shipment at liquid nitrogen temperatures which made sending the trays to other typing laboratories impracticable and therefore this aspect of the program was abandoned. Such trays maintained at liquid nitrogen temperatures remain a valuable tool for screening and standardizing tissue typing antisera.

The contract with Scripps Clinic to produce HLA xenoantisera will be allowed to expire. Although it initially showed promise and a few good typing reagents were developed there was poor reproducibility or consistency obtained with the methods being employed. This program element must await the future development of appropriate technology through grant-supported research.

The Jackson Laboratory continues its relationship to these programs as contractor for the production, storage, quality control, and distribution of mouse H-2 alloantisera to qualified investigators. This program was designed to provide immunologists and immunogeneticists with high-quality, standardized mouse H-2 histocompatibility antisera for their research on cellular and humoral immunity, graft rejection, tissue compatibility, diseases of tissue degradation and oncogenesis in the mouse model. The mouse immunogenetic system and its corresponding tissue antigens react similarly to the HLA system of man and therefore is of basic interest in enhancing our knowledge of the immune mechanism of humans.

During FY 76, 230 scientists used the mouse H-2 alloantisera reagents. Twenty-eight H-2 antisera are now being distributed (seven of which are being reproduced in 100 ml quantities) and nine additional antisera are being assayed prior to release. In addition, this contractor produces a very limited selection of non-H-2 antisera.

A contract was awarded during the last quarter of FY 76 to Washington University for the production of mouse antisera specific for antigens located in the mouse I region. Initial efforts will be directed at the production of antisera specific for antigens Ia 1, Ia 3, Ia 5, Ia 7, and Ia 8. These antigens have been identified as being important determinants for the immune response in mice and will provide researchers in this area which critically needed reagents.

II. Infections

A. Nucleic Acid Recombinants-Biotechnology

During the last two years the technology for the cloning of heterologous DNA in prokaryotic organisms has been developed to an advanced degree. This technology has the potential of being applied to the solution of a wide variety of problems of current interest to biomedical researchers in many disciplines and the potential benefits have been widely discussed in both the public and scientific literature. There are, however, potential risks associated with the indiscriminate use of the technology, and primarily these revolve around the inadvertent introduction of toxins or other virulence factors into the prokaryotic host and their subsequent escape from the laboratory and infection of natural hosts including man. Physical containment practices are in rather widespread use in laboratories doing work with pathogenic microbes. Physical containment procedures are, however, not foolproof and there remains a potential biohazard if equipment failure occurs or there is a breach in technique. At the Asilomar Conference in February 1975, the concept of biological containment was developed and is based on the presumption that vectors and hosts for DNA cloning can be constructed that are absolutely dependent on carefully controlled laboratory conditions for viability. Such vectors and hosts would "self-destruct" once removed from the specifically designed environments and, therefore, not present a biohazard to the community.

The staff of RRB has been working with members of the NIH Recombinant DNA Molecule Program Advisory Committee throughout FY76 in structuring a program aimed at the construction and testing of such safer vectors and hosts. Work will be initiated during the final two months of FY76 subsequent to the negotiation and award of five contracts in this new area. Two contractors will be producing safer bacteriophage vectors for E. coli hosts and a single contractor will produce plasmid vectors for this same bacterium. Two other contractors will produce safer E. coli hosts for these vectors. It is anticipated that this program will result in some of these safer systems being available for distribution in approximately one year.

B. Research Resources - Research Reagents

1. Viral Reagents

This program area was the primary emphasis of RRB during its first seven or eight years of activity and, therefore, the initial program objectives are rapidly nearing completion. Prior years of intensive work have resulted in completed work on the enteroviruses, adenoviruses, rhinoviruses, myxoviruses, and the agents of hepatitis A and B. In most cases seed virus preparations and corresponding antisera are now available for the viruses of interest, and it is anticipated that only rarely will new reagents in these categories be required as new serotypes are discovered and described.

During FY76 the one remaining contractor for the production of arbovirus reagents at the California State Department of Health Laboratories completed all specified work. This collection now consists of more than 80 reagent sets and contains seed virus and antibody preparations for arboviruses of health or health research significance. The arbovirus certification laboratory is located at Yale University and serves as an independent source of data on acceptability of reagents and provides a complete characterization of reagents produced by other contractors. It is expected that the certification laboratory will complete all work with these reagents during the coming fiscal year.

The contractor at St. Jude Children's Hospital is nearing completion of work aimed at producing monospecific antisera against all the hemagglutinins, neuraminidases, ribonucleoproteins and the matrix proteins of the influenza viruses of man and animals. Only some very difficult anti-neuraminidases remain to be produced and all work on this important project will be completed during the first quarter of FY77. These reagents will be a valuable resource in identifying new or unusual serotypes of this important human pathogen such as the recent isolates of swine influenza virus from human cases.

RRB is presently reviewing proposals to establish a Viral Testing Laboratory to service this total program area. The Branch needs a facility to verify feedback data from recipients concerning storage stability, activity and viability of the various reagents in this program. It is anticipated that a contract award will be made at the end of FY76 or very early in the Transition Quarter.

2. Processing and Distribution

In addition to the various program elements detailed above, the Research Resources Branch also distributes coronaviruses, herpesviruses, interferons, mycoplasmas, reoviruses and rhinoviruses.

The processing contract for all reagents in this division remains with the American Type Culture Collection (ATCC) and work is proceeding at an optimal rate. During the past year the ATCC has completed the processing of 65 reagent lots. This contract is a critical part in the Research Resources Branch program since the expertise of the processing laboratory is relied on to prevent degradation of reagent quality during final processing and packaging.

The Research Resources Branch reagent collection (exclusive of transplantation reagents) now includes over 890 individual reagents. The repository and distribution contract remains at Flow Laboratories, Rockville, Maryland; a tabular record of distribution by this facility since FY70 is as follows:

Fiscal Year	Total Transactions*	Total amps. & vials Distributed
1970	474	26,242
1971	638	12,902
1972	572	21,801
1973	575	19,181
1974	500	9,932
1975	592	6,751
1976 (9 months)	595	7,522

*Does not include the shipments made for testing and packaging purposes.

The following two (2) tables describe the distribution of reagents by groups and also by institutional affiliation during the first three quarters of FY 76.

RESEARCH RESOURCES BRANCH
DISTRIBUTION OF SPECIFIC VIRUS GROUPS
July 1, 1975 - March 31, 1976

# of Items Avail. (% of Inventory)	Virus Group	SHIPMENTS # of	SHIPMENTS % of	AMPOULES # of	AMPOULES % of # Distributed
70 (7.9)	Adenovirus	27	4.5	734	9.8
4 (.4)	Allergen	54	9.1	503	6.7
204 (22.9)	*Arbovirus	42	7.1	534	7.1
231 (25.9)	**Enterovirus	97	16.3	1876	24.9
9 (1.0)	Hepatitis	110	18.5	497	6.6
18 (2.0)	Herpes	10	1.7	43	.6
4 (.4)	Interferon	57	9.6	120	1.6
86 (9.7)	Mycoplasma	40	6.7	703	9.3
79 (8.9)	Myxovirus Paramyxovirus and related	74	12.4	421	5.6
6 (.7)	Reovirus	6	1.0	24	.3
180 (20.2)	Rhinovirus	9	1.5	203	2.7
	Combinations	69	11.6	1864	24.8

Figures based on 595 shipments and 7522 ampoules shipped (excludes testing, packaging and reagents transferred to ATCC).

*Included in this figure are 17 shipments of Arbovirus Grouping fluids totaling 321 ampoules.
**Included in this figure are 46 shipments of Enterovirus Typing Pools totaling 1313 ampoules.

RESEARCH RESOURCES BRANCH
FY 1976 SHIPMENTS
JULY 1, 1975 - MARCH 31, 1976

TYPE OF ORGANIZATION	SHIPMENTS TO GRANTEES	ITEMS SHIPPED	SHIPMENTS TO CONTRACTORS	ITEMS SHIPPED	OTHER SHIPMENTS	ITEMS SHIPPED	TOTAL SHIPMENTS	TOTAL ITEMS
UNIVERSITIES	40	522	31	235	102	944	173	1701
COMMERCIAL	0	0	4	28	49	255	53	283
HOSPITALS	14	229	9	67	33	372	56	668
FOUNDATIONS (NON-PROFIT)	5	44	20	316	13	59	38	419
STATE OR CITY HEALTH LABS	0	0	5	56	25	287	30	343
OTHER FEDERAL AGENCIES	0	0	0	0	34	1103	34	1103
NIH	0	0	0	0	35	293	35	293
FOREIGN	0	0	0	0	176	1911	176	1911
WHO	0	0	0	0	2	810	2	810
REAGENT TESTING	0	0	0	0	32	1667	32	1667
BULK FOR PACKAGING	0	0	0	0	10	0	10	0
DISCARD	0	0	0	0	0	0	0	0
ATCC TRANSFER	0	0	0	0	39	27374	39	27374
TOTALS	59	795	69	702	550	30461	678	28964

The preparation of the 1975-77 edition of the NIAID Catalog of Research Reagents was completed by RRB staff and was printed by the Government Printing Office (GPO). The new catalogs were received from the GPO in August 1975 and subsequently over 1300 copies of the catalog have been mailed out to various laboratories and individual scientists. The new catalog has greatly enhanced the value of the reagents by supplying new and updated data as well as describing approximately 150 new reagents which had not been included in the previous edition of the catalog. As indicated in the reagent distribution tabulations, the increased number of shipments can be attributed to the availability of the new catalog.

The Branch has continued its efforts to publicize the availability of these valuable reagents to the scientific community. In collaboration with the NIAID Office of Research Reporting and Public Response, the Branch took exhibits describing the reagents program to two scientific meetings. The first exhibit was taken to the American Academy of Allergists meetings convened in San Juan, Puerto Rico in March 1976. The second exhibit was shown at the American Society for Microbiology meetings in Atlantic City, New Jersey in May 1976. The exhibits were well received at both meetings and a great deal of interest was expressed by those individuals especially by those who were unaware of the NIAID reagents program.

After a category of reagents has become established, and the area of research that it supports is well defined it is of questionable value for RRB to continue storing and distributing that category of reagents for a prolonged period of time. During FY75 it was determined that four viral reagent groups fall into this category and RRB entered into a five year contractual arrangement with the American Type Culture Collection (ATCC) to transfer the enterovirus, adenovirus, rhinovirus and arbovirus reagent collections to the ATCC whose prime function is to store and distribute these types of reagents.

Under the contractual arrangement with ATCC, samples of each reagent type will be preserved as a museum function. Also the viability or homologous antibody activity of a parallel collection of reagents will be assayed. After successful completion of the first cycle of assay for each group, RRB will discontinue that reagent group from its catalog with a notation that they are available from ATCC and all remaining stock of that group of reagents will be transferred to ATCC for its custodianship. NIH scientists and NIAID contractors will be provided reagents without cost until RRB supply is depleted.

During this initial year of the 5-year contract, 50 samples of each reagent type for the four viral reagent groups have been transferred for the museum function. The assay of the enterovirus group has been conducted during this period and is nearing completion. After the test results from this assay have been reviewed, the total monospecific enterovirus reagent collection will be transferred to ATCC.

3. Molecular Anatomy Program

A major project of the Molecular Anatomy Program in FY 76 was the preparation of two monovalent vaccines for Type B hepatitis. The 22-nm form of HBsAg was purified by a three-step zonal centrifuge procedure from the plasma of chronic HBsAg carriers. Approximately 51 liters of human plasma were processed to prepare 522-mg of HBsAg/adw and 464-mg of HBsAg/ayw respectively. Bulk concentrates (200 µg of HBsAg protein/ml) of each candidate vaccine were inactivated with formalin for 96 hours at 37°C and stored frozen at -80°C. Safety and efficacy with HBsAg/adw vaccine in the chimpanzee model system are currently in progress; similar studies with the HBsAg/ayw vaccine will begin in May, 1976.

Further definition of the immunochemistry of HBsAg was made in FY 76. Direct antigenic analysis of the isolated major polypeptides of HBsAg demonstrated that they shared antigenic determinants. The major polypeptides of HBsAg/adw all contained a and d specificities as part of their constituent structure.

Additional characterization of the Dane particle, its DNA polymerase product, and the core was achieved this year. The core (HBcAg) was purified from extracts of HBV-infected human liver and radiolabelled with ^{125}I. The core was shown to contain two constituent polypeptides. The HBsAg-specific DNA polymerase was evaluated as a marker of HBV infectivity in a retrospective analysis of donor-recipient pairs in the clinical trials with hyperimmune gamma globulin. The correlation between polymerase in the HBsAg-positive donor and Type B hepatitis in the recipient was highly significant and indicates that the level of polymerase in serum is a good indicator of HBV infectivity. DNA polymerase in the sera of chronic-carrier chimpanzees was monitored in a study of the role of interferon in alteration of the chronic carrier state. In a preliminary experiment endogenously-induced interferon in the serum correlated with a reduction in serum polymerase levels.

Basic studies of the biochemistry and immunochemistry of Respiratory Syncytial virus (RSV) and use of the radiolabelled ribonucleo-protein (RNP) in tests for anti-RNP in serum on nasal wash specimens were accomplished.

Part A

Individual Contract and Agreement Tabulation

PROGRAM: IMMUNE SYSTEM AND DISEASE - ALLERGEN REAGENTS BRANCH: RRB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Flora, Robert AI 52516 Worthington Biochem. Prior Year Funds	To extract and package the group antigens I, II, and III from 9 kilograms of ryegrass (Lolium perenne) pollen.
Gleich, Gerald AI 42546 Mayo Clinic $35,000	This facility serves as the certification laboratory for the Allergen Reagents Program. Bulk products are assayed to ascertain if they meet contract specifications and final packaged materials are tested for potency and are fully characterized.
Goodman, Howard AI 52503 World Health Org. Prior Year Funds	To acquire in collaboration with the International Union of Immunological Societies on International Standard reagent for fluorescently labelled anti-human IgM
Heiner, Douglas AI 42547 Harbor General Hosp. Prior Year Funds	To produce an anti-human IgE antiserum for use in the radioallergosorbant test for assaying the allergic status of individuals
Strejan, Gill AI 42500 Univ. Western Ontario Prior Year Funds	To produce 500 mg of the allergen Asc-1 from Ascaris suum

Individual Contract and Agreement Tabulation

PROGRAM: IMMUNE SYSTEM AND DISEASE - REAGENTS AND TECHNICS

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Adkins, Harry AI 22515 $184,270	Maintain a repository for processing and distribution of NIAID reagents. To provide laboratory space and a warehouse facility to receive, process, store and ship reagents. This contractor made 1,554 shipments of bulk antisera and NIAID tissue typing trays to 371 world-wide investigators. Thirty-one liters of antisera were received and processed.
Braun, William E. AI 81298 Cleveland Clinic $121,744	Operate a regional leucocyte phenotyping facility, to evaluate tissue typing techniques; to match donors and recipients in kidney and bone marrow transplants so as to be able to ascertain their degree of immunologic identity by utilizing microlymphocytotoxicity typing and other cross matching procedures including mixed lymphocyte culture. Some observations are: escape from sensitization of normal and leukemic cells (possible diagnostic tool to determine leukemics), a severe but reversible rejection in an HLA identical, MLC non-stimulating renal allograft recipient was documented, HLA-BW17 and a similar D locus antigen was found to be associated with renal cell carcinoma, B cell sera were identified and LD typing cells are being accumulated. The contractor maintains a large reference panel of frozen cells which are well characterized and are used to test the quality of sera being obtained for the NIAID Serum Bank and to test the NIAID Serum Trays. This cell panel is also used to screen sera so as to be able to provide 5000 ml of rare typing antisera to the NIAID Serum Bank.

Cherry, Marianna
AI 12257
The Jackson Laboratory
Prior Year Funds

The production, storage, quality control and distribution of mouse H-2 alloantisera to qualified investigators. During FY 76 two non H-2 d or k alloantisera, Thy 1.1 and Thy 1.2 were released for distribution by the contractor, thus bringing the total antisera available for distribution to twenty-eight. Seven H-2 d or k antisera are in very low supply and the contractor is producing 100 ml each of these reagents. The contractor has produced nine additional antisera which are being quality controlled in her laboratory prior to release.

Ferrone, Soldano

Production of HLA xenoantisera. Under ordinary circumstances, typing sera used by the bank are obtained from multiparous woman. Many specificities are infrequently found in Caucasian population, therefore naturally occurring antibodies are difficult, if not impossible, to find. This contract was awarded to determine if antisera could be produced by the injection of soluble antigens into animals. Although a few good reagents were developed during the course of the contract a production protocol which would give consistant results was not developed. This contract will be allowed to expire June 27, 1976.

Perry, Vernon
AI 32508
American Foundation for Biological Research
$123,129

Freeze-drying of typing sera. To freeze-dry typing sera in a manner which completely preserves the typing reactivity of the reagent. During FY 75 approximately 117 liters of antisera were freeze-dried in 1 ml vials. All bulk reagents are now shipped at ambient temperatures and this has resulted in a considerable savings in the transportation charges incurred. The laboratory has determined the activity of over 1000 freeze-dried sera during the past year. A frozen lymphocyte tray was developed. It was discovered that the trays could not be removed from liquid nitrogen storage and shipped at higher temperatures without

killing the cells. However this procedure can be used by any laboratory who desires to prepare frozen lymphocyte trays for screening of sera or cross matching donor-recipient cells.

Shreffler, Donald
AI 62502
Washington University
$60,000

The production, packaging, storage, and quality control of mouse antisera pools which are specific for the mouse immune response (Ir) region antigens (Ia antigens). The Ia mouse antisera will be used by immunologists to enhance knowledge concerning the role of the immune response in relation to mouse H-2 histocompatible and histoincompatible transplanted tissue, and to the predilection and control of communicable and autoimmune diseases. The contractor is to produce 400 ml each of five antisera directed against Ia 1, Ia 3, Ia 5, Ia 7 and Ia 8 specificities.

Spees, Everett
AI 50003 (Interagency Agreement)
Walter Reed Army Medical Center
$153,663

Human histocompatibility and transplantation immunology quality control testing facility. The first contract year has been spent mainly in expanding their equipment base and searching for two technicians. The laboratory provided the technology and lymphocytes for cell exchange and cross matching exercises for the KTHS study. They are currently sending their cell panel to other quality control laboratories for refinement and confirmation. The second year should see them fully involved in the testing of the NIAID serum tray and bulk sera.

Trapani, Robert-John
AI 12443
Microbiological Associates, Inc.
$49,000

The contractor prepares and evaluates human histocompatibility typing trays. The typing of HLA determinants in human transplantation by using the best HLA antisera in the NIAID Serum Bank has resulted in the delivery to U.S. transplant centers of high quality 72-well typing trays whose results are statistically comparable from laboratory to laboratory. They aid in organ exchange programs and in the analysis of the efficacy of histocompatibility matching in organ graft survival. During the

FY 76 contract period this contractor produced approximately 34,000 trays which were quality controlled by independent laboratories prior to being released to the U.S. transplant centers.

van Rood, J.J.
AI 42508
University of Leiden
$99,641

To continue the search for HLA antisera and to improve histocompatibility testing techniques. The contractor operates a large local screening program and encourages other Eurotransplant centers to send antisera to the NIAID Serum Bank. The Leiden program produced approximately 5000 ml of much needed typing sera for the bank. Their work indicated that MLC inhibiting antibodies were detecting determinants present on B cells, but absent or present in far smaller amounts on T cells and platelets; the two stage complement dependent NIH technique was modified in such a manner that it recognized these antibodies. As a result of a study performed on the heterogeneity of HLA antigens, splits in the following antigens have been found BW16, BW15, BW21 and B12. A technique has been developed where by fluorescein diacetate (FDA) labeled cells can be automatically read with the microscope.

Individual Contract and Agreement Tabulation

PROGRAM: INFECTIONS - NUCLEIC ACID RECOMBINANTS - BIOTECHNOLOGY BRANCH: RRB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Blattner, Frederick R. AI 62506 Univ. of Wisconsin $45,000	Construction of safer lambda phage cloning vectors for E. coli hosts. The safer vectors will be able to propagate only in a unique laboratory adapted host and be unable to establish a prophage or carrier state. Thus, their dissemination in nature would be self-limiting.
Curtiss, Roy AI 62508 Univ. of Alabama $217,000	To construct a variety of E. coli hosts for both phage and plasmid vectors of DNA recombinant molecules. Such hosts will be dependent on specific laboratory controlled conditions for growth or survival and will thereby markedly improve the safety of the vector-host systems.
Helinski, Donald R. AI 62509 Univ. of California at San Diego $56,000	To develop safer plasmid vectors in support of DNA recombinant molecule research. Such vectors shall only replicate in unique laboratory adapted hosts and be non-conjugative and poorly or non-mobilizable.
Ray, Dan S. AI 62505 Univ. of California at Los Angeles $33,000	To construct a safer M13 phage cloning vector for DNA recombinant molecules. This safer vector will be able to propagate only in a unique laboratory adapted host, thus, its dissemination in nature will be self-limiting.
Wilcox, Gary AI 62507 Univ. of California at Los Angeles $31,000	To construct a safer E. coli host for M13 phage vector. This host will be dependent for growth on unique laboratory controlled conditions and be unable to transfer the resident vector to another bacterium.

Individual Contract Agreement Tabulation

PROGRAM: INFECTIONS - VIRAL REAGENTS BRANCH: RRB

Principal Investigator Contract Number Contractor FY 76 Obligations	Ojbectives and Findings
Emmons, Richard AI 80679 Cal. State Dept. of Public Health Prior Year Funds	The contractor is the sole producing contractor for arboviruses. During this terminal contract period he is producing reagents for Dengue 1 and 4, LCM, Bocas and Gumbo Limbo viruses.
Schmidt, Nathalie AI 90089 Cal. State Dept. of Public Health Prior Year Funds	This laboratory serves as a certification facility for enteroviruses. In addition to certification testing of bulk and final packaged materials produced by other contractors it is also involved in the identification of new types and storage stability tests.
Shope, Robert AI 42517 Yale University Prior Year Funds	This laboratory is the certification facility for the arboviruses. All reagents in this category are assayed in bulk and final packaged form to assure that they meet contract specifications, are potent, and completely characterized.
Webster, Robert AI 32516 St. Jude Hospital Prior Year Funds	This contractor is producing monospecific antisera against all the known subunits of the influenza viruses of man and animals. At this time he has produced antisera for all subunits except two neuraminidases

Individual Contract Agreement Tabulation

PROGRAM: INFECTIONS - PROCESSING AND DISTRIBUTION BRANCH: RRB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Adkins, Harry AI 72-2515 Flow Laboratories $166,000	When the reagents program was given the responsibility for production of reference reagents, other responsibilities were also included, i.e., the development and maintenance of appropriate means for the storage and distribution of these research materials and this contractor fulfills these responsibilities. During the past year, the contractor has been supplying the storage and shipping services for the RRB, the Geographic Medicine Branch, and the Infectious Diseases Branch. As a result of these combined activities, the contractor has made 1,120 shipments totaling 42,000 vials and 95,000 ml of bulk material. Approximately 70 incoming shipments have been handled totaling 60,692 vials and approximately 75,000 ml of bulk materials. (In addition see Immune System and Disease - Reagents and Technics)
Donovick, Richard AI 75-2526 American Type Culture Collection Prior Year Funds	After a category of reagents has become established and the area of research that it supports is well defined, it is of questionable value for RRB to continue storing and distributing that category of reagents. Therefore, in FY 75, RRB entered into a cooperative enterprise with ATCC to transfer the enterovirus arbovirus, rhinovirus and adenovirus reagent collections to the ATCC for their custodianship and distribution; complete transfer of the four viral reagents groups will be accomplished over a five-year period. During this first year of the contract, 50 samples of each reagent type for the four viral reagent groups have been transferred for

museum storage purposes. Under the terms of the contract each of the four collections of reagents is to be assayed for viability or homologous antibody before the complete transfer is made, therefore, the contractor has been assaying the enterovirus group during this initial period. The assay of this group is nearing completion and after the test results have been reviewed, the total monospecific enterovirus reagents collection will be transferred to ATCC.

Stevens, David A.
AI 74-2515
American Type Culture Collection
$52,000

The program goals of the RRB are to produce, test, package and distribute standardized research reagents to the scientific community. This contract performs all the packing services required for the Branch to accomplish its program goals. During the past year, the contractor processed a total of 65 reagent lots. After the processing is completed, samples of the final product are returned to the certification laboratory to verify that the ampouled contents as identified on the label are correct and to reverify titers. ATCC has been very careful in complying with the contract specifications which stipulate that only one reagent lot should be handled at a time in order to alleviate the possibility of mislabeling.

Individual Contract Agreement Tabulation

PROGRAM: INFECTIONS - MOLECULAR ANATOMY PROGRAM BRANCH: RRB

Principal Investigator Contract Number Contractor FY 76 Obligations	Objectives and Findings
Gerin, John 00002-07 Energy Research and Development Admin. $562,000	The major objective is to develop and apply methods of isolating viruses and viral antigens and the application of those techniques to research on associated diseases. During FY 76 this laboratory was involved in a variety of studies on hepatitis B but the most significant involvement was the central role of this facility in developing a hepatitis B vaccine. Application of techniques developed during prior years are being applied in this project. Other project areas investigated were: hepatitis B reagents, immunochemistry of hepatitis B polypeptides, isolation and characterization of antigenic components of respiratory syncytial virus, as well as serving NIAID as a training facility for intramural scientists in selected techniques.

Part B

I. Contract Publications

Alter, H.J., L.B. Seef, P.M. Kaplan, V.J. McAuliffe, E.C. Wright, J.L. Gerin, R.H. Purcell, and P.V. Holland. HBsAg-Specific DNA Polymerase and e Antigen as Indicators of Infectivity of Human Blood: Implications for Clinical Trials of Hepatitis B Immune Globulin. New Engl. J. Med. (Submitted, 1976).

Banowsky, L.H., Braun, W.E., Crile, G., Straffon, R.A. Deodhar, S., and Loening, S.: Transcervical thymectomy in renal transplant recipients-surgical complications and possible effect on long term allograft survival. Proc. of the American Society of Genito-Urinary Surgeons, 1975 (Abstract).

Banowsky, L.H., Braun, W.E., Magnusson, M.O.: The medical and legal determination of death--its effect on cadaveric organ procurement. Journal of Legal Medicine 2:44, 1974.

Banowsky, L.H., Francis J., Braun, W.E., Magnusson, M.O.: Lymphatic complications of renal transplantation. Urology 4:650, 1974

Banowsky, L.H., Montie, J.E., Braun, W.E., and Magnusson, M.O.: Prevention of wound infections in recipients of renal transplants. Urology 4:656, 1974.

Banowsky, L.H., Siegel, D.F., Hewitt, C.B., Stewart, B.H., Straffon, R.A., Magnusson, M.O., and Braun, W.E.: Increasing the supply of kidneys for transplantation: Use of donor organs with multiple vessels. Urology 4:643, 1974.

Belvedere, M. C., Cortoni, E. S., Dausset, J., Lamm, L. U., Mayr, W., Van Rood, J.J., Svejgaard, A. and Piazza, A. On the heterogeneity of linkage estimations between LA and Four loci of the HLA system. Tissue Antigens 5, 2: 99-102, 1975.

Bijnen, A.B., Schreuder, Ieke, Giles, C.M., Los, W.R.T., Meera Khan, P., Volkers, W.S. and Van Rood, J.J. A study on genetic markers in families with a recombination in the HLA region. In 2nd Int. Conference on Human Gene Mapping, Rotterdam June 1974. Birth Defects: Original Article Series, New York, National Foundation 1974/75 in press.

Braun, W.E. and Kayhoe, D.E.: Screening sera for HL-A antibodies, in Manual of Clinical Immunology, E.J. Yunis, ed. In press.

Braun, W. E., Strimlan, C. V., Straffon, R. A., Zachary, A. A. Bartee, S. L., and Grecek, D.R.: The association of W17 with familial renal cell carcinoma. Tissue Antigens. In press.

Bruning, J.W. Serologic recognition of the histocompatibility antigens using agglutination and cytotoxicity techniques. Sem. Hemat. 11, 3:263-271, 1974.

De Jong-Bakker, M., Cleton, F.J., D'Amaro, J., Keuning, J.J. and Van Rood, J.J. HL-A and breast cancer, Europ. J. Cancer 10, 9: 555-594, 1974.

Dvorak, K.J., Braun, W.E., Stowe, N.T., Magnusson, M.O., and Banowsky, L.H.: Effect of high doses of methylprednisolone on the isolated, perfused kidney. Transplantation, In press.

Ferrone, S.: Natural Antibodies to Human Lymphocytes in Murine Strains, Journal of the National Cancer Institute, 1976, Folder #24, 419

Geerts, S.J., Pottgens, H., Limburg, M. and Van Rood, J.J. Predisposition for atopy or allergy linked to HL-A. (Letter to the Editor) Lancet i, 7904: 461, 1975.

Gerin, J.L. The Dane Particle in Type B Hepatitis, In: Transmissible disease and Blood Transfusion (eds.) T.J. Greenwalt and G.A. Jamieson, Grune and Stratton, New York, p. 125-128 (1975).

Gerin, J.L. Hepatitis: The Search for Viral and Sub-viral Antigens. Fractions, 1:1-9 (1976).

Gerin, J. L., R. M. Faust, and P. V. Holland. Biophysical Characterization of the adr Subtype of Hepatitis B Antigen and Preparation of Anti-r Sera in Rabbits. J. Immunol., 115: 100-105 (1975).

Gerin, J.L., E.C. Ford, and R. H. Purcell, Biochemical Characterization of Australia Antigen: Evidence for Defective Particles of Hepatitis B Virus. Amer. J. Pathol. 81: 651-668 (1975).

Gerin, J.L., J. W-k. Shih, and P.M. Kaplan. Biophysical and Biochemical Characterization of Hepatitis B Antigen. Amer. J. Med. Sci., 270:115-121 (1975).

Giphart, M.J., Doyer, E., Wisse, E. and Bruning, J.W. Quantitative aspects of HL-A 2 antigenic surface determinants studied with radiolabelled antibodies. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Giphart, M.J., Doyer, E. Bruning, J.W. and Van Rood, J.J. HL-A antibodies. J. Immunol. Methods 1975 in press.

Gold, J.W.M., J. W-K. Shih, R.H. Purcell, and J.L. Gerin. Characterization of Antibodies to the Structural Polypeptides of HBsAg: Evidence for Subtype Specific Determinants: J. Immunol. (Submitted, 1976).

Goulmy, E., Termijtelen, A., Keuning, J.J. and Van Rood, J.J. CML reaction between unrelated SD and LD identical individuals. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Grecek, D.R., Braun, W.E., and Bartee, S.L.: Escape from sensitization of normal and leukemic lymphocytes. Proceedings of the American Association for Clinical Histocompatibility Testing, Birmingham, Alabama, 1975 (Abstact)

Haverkorn, M.J., Hofman, B., Masurel, N. and Van Rood, J.J. HL-A linked genetic control of immune response in man. Transpl. Reviews 22: 120-124, 1975.

Hoffman, E.J., E.C. Ford R.M. Chanock, and J.L. Gerin. A Radioimmunoassay for the Ribonucleoprotein of Respiratory Syncytial Virus. Abstr., Amer. Soc. Microbiol. May, 1976.

Jonker, M., Persijn, G. G. and Van Rood, J. J. MLC inhibition studies with sera from multiparous women. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Juji, T., Hagino, Y., Tamura, M., Ohtawa, T., Toyama, H., Shibata, Y., Schreuder, Ieke and Van Rood, J. J. Absorption study on cross-reactivity between W22 and J-1 antigens. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Kaplan, P. M., E. C. Ford, R. H. Purcell, and J. L. Gerin. Demonstration of Subpopulations of Dane Particles. J. Virol., 17:885-893 (1976).

Kapikian, A. Z., A. R. Kalica, J. W-K. Shih, W. Lee Cline, T. S. Thornhill, R. G. Wyatt, R. M. Chanock, H. W. Kim, and J. L. Gerin. Buoyant Density in Cesium Chloride of the Human Reovirus-like Agent of Infantile Gastroenteritis by Ultracentrifugation, Electron Microscopy, and Complement Fixation. Virology, 70:564-569 (1976).

Keuning, J. J., Termijtelen, A., Blusse van Oud Alblas, A., Van den Tweel, J.G.,Schreuder, Ieke and Van Rood, J. J. Typing for MLC (LD). Transpl. Proc. VII: 35-39, 1975.

Keuning, J. J., Termijtelen, A., Blusse van Oud Alblas, A., Gabb, B. W., D'Amaro, J. and Van Rood, J. J. LD (MLC) Population and Family Studies in a Dutch Population. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Keuning, J. J., Van den Tweel, J. G., Gabb, B. W., Termijtelen, A., Goulmy, E., Blokland, E., Elferink, B. G. and Van Rood, J. J. An estimation of the recombination fraction between the MLC locus and the Four locus. Tissue Antigens 1975 in press.

Khanna, R., Braun, W. E., Lewis, L., and Banowsky, L. H.: Lipid abnormalities in patients with stable chronic renal failure (CRF), chronic intermittent dialysis (CID), and renal transplant (RT). Proceedings of the 6th International Congress of Nephrology, Florence, Italy, 1975, (Abstract). In press.

Kroener, C. A., Perry, V. P.,Martin, J. L. and Sasso, J. C. "Freeze Drying of Histocompatibility Typing Sera" Cryobiology 12: 397-404; 1975.

Loening, S., Banowsky, L. H., Braun, W. E., and Magnuson, M. O.: Bladder neck contracture and urethral stricture as a complication of renal transplantation. J. Urol. In press.

McCoy, G. C., Loening, S., Braun, W. E., Magnusson, M. O.,Banowsky, L. H., and McHenry, M.C.: The fate of cadaver renal allografts contaminated before transplantation. Transplantation. In press.

Perry, V. P., Martin, J. L. and Kroener, C. A. "Nutrient Medium and Frozen Storage of Human Lymphocytes: Cryobiology 12: 386-396; 1975.

Purcell, R. H. and J. L. Gerin. Hepatitis B Subunit Vaccine: A Preliminary Report of Safety and Efficacy Test in Chimpanzees. Amer. J. Med. Sci., 270: 395-399 (1975).

Richiardi, P., Castagneto, M., D'Amaro, J., Schreuder, Ieke, Vassali, P. and Curtoni, E. S. Four new HL-A allelic factors subtypic to HL-A 12 and W15. Their correlation with W4 and W6. J. Immunogenet. 1: 323-335, 1974.

Roberts-Thomson, I. C., Stevens, D. P., Braun, W. E., Morris, P. J., Michel, B., Wall, A. J., Dworken, H. J.: HL-A type and small bowel changes in dermatitis herpetiformis. Gastroenterology 1975 (In press) (Abstract)

Schmidt, R. N., Lake, R. B., Braun, W. E.: Monte Carlo simulation of a regional kidney matching program. Proceedings of the 5th Conference of the Canadian Medical and Biological Engineering Society, September, 1974 (Abstract)

Schnitzer, T. J., D. S. Hodes, J. L. Gerin, E. Camargo, and R. M. Chanock. Effect of 2-deoxy-D-glucose and Glucosamine on the Growth and Functions of Respiratory Syncytial and Parainfluenza 3 Viruses. Virology, 67: 306-309 (1975).

Schreuder, Ieke, Van Leeuwen, A. and Balner, H. A further study on the detection of HLA-like antigens in a colony of chimpanzees. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Schreuder, Ieke, D'Amaro, J., Sandberg, L. and Van Rood, J. J. Partition of the antigen W16 into two subtypic determinants. Tissue Antigens 5, 2: 142-144, 1975.

Shih, J. W-K. and J. L. Gerin. Immunochemistry of Hepatitis B Surface Antigen (HBsAg): Preparation and Characterization of Antibodies to the Constituent Polypeptides. J. Immunol., 115: 634-639 (1975).

Shih, J. W-K., P. L. Tan, and J. L. Gerin. Antigenicity of the Constituent Polypeptides of Hepatitis B Surface Antigen. Abstr., Amer. Soc. Microbiol., May 1976.

Van den Tweel, J. G., Vriesendorp, H. M., Termijtelen, A., Westbroek, D. L., Bach, M. L. and Van Rood, J. J. Genetic aspects of canine mixed leukocyte cultures. J. Exp. Med. 140, 3: 825-836, 1974.

Van den Tweel, J. G., Vriesendorp, H. M.,Termijtelen, A., Westbroek, D.L., Bach, M. L. and Van Rood, J. J. Genetics of mixed luecocyte cultures in dogs. Transpl. Proc. VII: 155-158, 1975.

Van der Does, J. A., Van Leeuwen, A., Jeannet, M., Nijenhuis, L., Van Loghem, E., Meera Khan, P., Bernini, L., Bohnhof-Pallfy, Volken, B., Gabb, B. W. and Van Rood, J. J. HL-A typing in an inbred population in a Swiss Alpine village: Fieschertal. J. Immunogenet. 1975 in press.

Van der Linden, J.M.J.P., Keuning, J.J., Wuisman, J.H.C., Cats, A. and Van Rood, J.J. HL-A 27 Ankylosing spondylitis. (Letter to the Editor) Lancet i, 7905: 520, 1975.

Van Leeuwen, A., Winchester, R. and Van Rood, J.J. Serotyping for MLC. II. Technical aspects. Ann. N.Y. Acad. Sci, 254: 289-295, 1975.

Van Rood, J.J. The impact of the major histocompatibility complex on graft survival and disease susceptibility. Genetics 79: 277-291, 1975.

Van Rood, J.J. Logistics of organ acquisition. In: The current status of cardiac surgery, Fifth Cardiac Surgery Course London April 1st 1974. Lanchaster, Medical and Technical Publ. Co. Ltd., 1975 in press.

Van Rood, J.J. Serotyping for LD. In: Seventh Int. Course on Transplantation and Clinical Immunology, Lyon May 12-14, 1975, in press.

Van Rood, J.J., Blusse van Oud Alblas, A., Keuning, J.J., Frederiks, E., Termijtelen, A., Van Hooff, J.P., Pena, A.S. and Van Leeuwen, A. Histocompatibility genes and transplantation antigens. Concluding remarks. Transpl. Proc. VII: 25-30, 1975.

Van Rood, J.J., Van Hooff, J.P. and Keuning, J.J. Disease predisposition, immune responsiveness, and the fine structure of the HL-A supergene. A need for reappraisal. Transpl. Reviews 22: 75-104, 1975.

Van Rood, J.J., Van Leeuwen, A., Keuning, J.J. and Termijtelen, A. Serotyping for MLC. III. Family and population studies with an MLC inhibiting serum P1. Transpl. Proc. VII: 31-34, 1975.

Van Rood, J.J., Van Leeuwen, A., Keuning, J.J. and Blusse van Oud Alblas, A. The serological recognition of the human MLC determinants using a modified cytotoxicity technique. Tissue Antigens 5, 2: 73-79, 1975.

Van Rood, J.J., Van Leeuwen, A., Parlevliet, J., Termijtelen, A. and Keuning, J.J. LD typing by serology. IV. Description of a new locus with three alleles. In: Histocompatibility Testing 1975. Copenhagen, Munksgaard, in press.

Wernet, P., Winchester, R., Kunkel, H.G., Wernet, D., Giphart, M., Van Leeuwen, A. and Van Rood, J.J. Serological detection and partial characterization of human MLC determinants with special reference to B-cell specificity. Transpl. Proc. VII: 193-200, 1975.

Zachary, A.A., Fuller, T. C., and Braun, W. E.: Linkage disequilibrium between W29 and HL-A12. Proceedings of the American Association for Clinical Histocompatibility Testing, Birmingham, Alabama, 1975 (Abstract)

II. Staff Publications

Cunningham, S., and Nutter, J., (Eds): NIAID Catalog of Research Reagents, U.S. Dept. of Health, Education and Welfare, National Institutes of Health, Bethesda, Maryland, DHEW Publication No. NIH 75-899, 1975, 999 pp.

Hare, D. B., Kayhoe, D.E., and Ray, J. G., Jr., (Eds): Catalog of Tissue Typing Antisera, U.S. Dept. of Health, Education and Welfare, National Institutes of Health, Bethesda, Maryland, DHEW Publication No. NIH 76-141, October 1975, 237 pp.

Part C Workshops

1. Workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules.

Approximately 60 scientists from the U.S. and several foreign countries participated in this workshop held in La Jolla, California on December 1 to 3, 1975. The workshop was sponsored by this office. The purposes of the meeting were the exchange of recent data on the development of safer prokaryotic host-vector systems, devising methods of testing the level of containment provided by these systems and exploring the various directions that future research should take in the construction of safer bacterial systems for the cloning of foreign DNA.

2. First HLA Workshop of the Americas.

Partial support was provided to the First HLA Workshop of the Americas by funding the HLA Serum Committee meeting at NIH in September 1975; receiving, collecting and shipping the 284 sera in the first serum exchange which were screened by 31 laboratories, and publishing and mailing the proceedings of the First Workshop of the Americas, held in Los Angeles, December 1975, to over 400 worldwide laboratories.

3. The Second Annual Meeting of the American Association of Clinical Histocompatibility Testing.

The American Association of Clinical Histocompatibility Testing was awarded a contract to provide funds for administrative, logistics, and travel support for the planning and conduct of the second annual meeting of the AACHT, April 26th-29th, 1976 in New Orleans, Louisiana.

4. Workshop on the Antigens in Hypersensitivity Pneumonitis.

The Branch will sponsor this workshop on June 3 and 4, 1976. This workshop was originally recommended by the RRB Advisory Committee in June 1973. The information obtained from the workshop will be used as a basis to decide if reagents for this disease complex should be produced as part of the Allergen and Immunologic Reagents Program.

INFECTIOUS DISEASE BRANCH

The Infectious Disease Branch (IDB) supports research efforts which are designed to develop materials and methods which will significantly improve our ability to control major infectious diseases in the United States. To a large extent, these efforts result in the application of basic research findings to clinical medicine. While a majority of projects supported by the IDB involve the utilization of currently available research tools and techniques, support is also provided for a limited number of basic studies needed to fill critical scientific gaps in existing programs. The IDB contracts involve research efforts in six program areas: Sexually Transmitted Diseases, Hepatitis, Influenza, Other Viral Respiratory Infections, Bacterial Diseases and Antiviral Substances.

I. Sexually Transmitted Diseases - Gonorrhea and Syphilis

The venereal diseases represent a major infectious disease public health problem in the United States. During a workshop sponsored by the IDB in July, 1973, a number of research areas in venereal diseases were identified as being appropriate for the contract mechanism. Included were studies on the cultivation of Treponema pallidum, the causal organism of syphilis, and studies of the antigens of Neisseria gonorrhoeae directed, in particular, toward the development of a serologic typing system for the organism and candidate vaccine preparations.

Contracts were awarded during FY74 to the University of California at Los Angeles, Stanford Research Institute and the University of Minnesota-Hormel Institute for studies directed toward in vitro cultivation and propagation of the syphilis spirochete with emphasis on the use of modern tissue culture techniques. These contractors have made considerable progress in defining the optimum tissue cell lines and tissue culture medium for maintenance of motility and virulence of organisms grown in rabbit testis. Although multiplication of the organisms has not been documented, each of the contractors has been able to maintain motility and virulence of the organism for significantly longer periods of time than has been the experience in the past. In addition, new procedures have been developed for the transport of organisms from syphilitic lesions to the laboratory, for purification of rabbit grown organisms on ficoll gradients, and for the preparation of radioactively labeled DNA to be used in studies of genetic relationships within the genus Treponema. The contractors, using somewhat different techniques, have confirmed reports in the literature which indicate that the pathogenic treponemes are capable of utilizing oxygen and are probably not anaerobes as has been accepted. This observation may be a significant factor in the eventual development of procedures for cultivation.

Three contracts for studies of gonorrhea were awarded at the end of FY75. These efforts are primarily directed toward the development of a serologic typing system for the gonococcus. It is hoped that a better understanding of the organism's antigenic structure and a practical serologic typing system will result from these studies. Not only would such a typing system be valuable in epidemiological studies of gonorrhea and assist in the diagnosis of asymptomatic infection, but it would also provide information essential for future

development of gonococcal vaccines. Contractors at the University of Texas at Dallas, University of Washington, and the University of Pittsburgh have been able to show antigenic heterogeneity in both pilus preparations and preparations of major outer membrane protein from gonococcal strains. Using the major outer membrane protein antigen, the University of Texas contractor has established 16 serotypes while 12 "auxo-serotypes" (combination of pilus serotyping and auxotyping) have been proposed by the University of Pittsburgh contractor. Further characterization of the pili and animal studies directed at their antigenicity could lead to an appropriate candidate for a gonococcal vaccine.

II. Hepatitis

The mission of the hepatitis program is to support research activities leading to the control of hepatitis A, B and non-A, non-B virus infections. Major emphasis continues on studies of hepatitis B virus (HBV) infection since knowledge of the characteristics of HBV surface and core antigens (HB_SAg, HB_CAg) and antibodies (anti-HB_S, anti-HB_C) have made it possible to identify and study this disease. The projects supported have resulted from careful Institute planning, which included a series of workshops. Although the virus causing hepatitis A has been identified, progress in research on this agent has been limited by the unavailability of Sagiunus mystax marmosets. It has been demonstrated that these animals are the most suitable model for studies of hepatitis A pathogenesis and host serologic response. The NIAID, CDC, and BoB/FDA have entered into a collaboration to establish a breeding colony for S. mystax. Since an embargo has been imposed on the export of these animals from South America, it has been necessary for the collaborating organizational units to support trapping expeditions in Brazil. It is hoped that by the fall of 1976 100 pairs will be in the breeding colony.

The investigations of causative agents of viral hepatitis and measures for their control are collaborative efforts of intramural scientists, NIAID contractors, and other cooperating government agencies.

A. Vaccine Development and Testing

Last year's report described the preparation of monotypic HBV vaccines (subtypes adw and ayw) consisting of 22 nm surface antigens (HB_SAg). Approximately 10,000 doses (50 µg/dose) of each subtype vaccine were prepared, starting with approximately 100 liter amounts of human plasma containing HB_SAg. The vaccine was prepared by the Molecular Anatomy Laboratory (MAN) under contract with the Research Resources Branch.

Before these vaccines can be tested in man, they must be thoroughly investigated for safety, antigenicity, and efficacy in the chimpanzee animal model. Using these animals, efficacy trials are currently underway with the adw vaccine and trials with the ayw vaccine should start in the near future. Seronegative chimpanzees for the studies have been obtained primarily from the Holloman Air Force Base chimpanzee breeding colony which, for the past four years, has been jointly funded by the NIAID-BoB-CDC. The trials are being conducted in two

animal holding and research facilities under contract with the NIAID. In addition, the CDC laboratory in Phoenix, Arizona, is providing facilities and staff for part of the testing.

These studies should answer numerous questions relating to vaccine safety. Is the vaccine free of infectious virus? Do animals possessing HB_SAg or anti-HB_SAg show any adverse reactions to the vaccine? In addition to safety, numerous questions relating to level of antibody response, cross-protection of different subtypes, duration of immunity, and cellular immune factors will be actively investigated. Contractors of the NIAID and intramural scientists will be participating in the various immunologic and pathologic examinations of materials from the test animals.

The NIAID is currently evaluating the hepatitis B vaccines as monovalent preparations. During the past year, a contract was awarded to the New York Blood Center to test a bivalent vaccine (mixture of adw and ayw) in chimpanzees. This activity represents a collaborative effort of the NIAID and the New York Blood Center (NYBC). The NYBC had planned development and evaluation of vaccines similar to the NIAID effort and had established a chimpanzee colony in Liberia, Africa. In order to avoid duplication of effort and conserve animal resources, the NYBC and NIAID will jointly support studies of safety, antigenicity, and efficacy of the NIAID bivalent vaccine in chimpanzees at the Liberian colony.

The NIAID is evaluating the hepatitis B vaccines in a stepwise fashion. Sufficient vaccine is available for human testing if all animal work indicates safety, antigenicity and efficacy. The first humans to receive such a vaccine should be individuals not "at risk" for hepatitis B infection. If "at risk" volunteers were employed it would be virtually impossible to resolve HBV infections due to intercurrent infection or possible infectiousness of vaccine. Since the incubation period for HBV infections can be as long as six months, it is desirable to utilize cloistered populations such as the Trappist monks for these trials. During the past year the IDB has visited monasteries and has received favorable replies for participation in future trials.

Once the safety and antigenicity of the vaccine has been established in volunteers, it will be desirable to determine efficacy in populations at "high risk" for HBV infections. Evidence suggests that staff and patients of hemodialysis units have a high attack rate for HBV and would represent a population where vaccine efficacy could be determined. However, before trials commence, it will be necessary to obtain baseline epidemiologic data to assess true annual attack rates. The IDB is in the process of receiving proposals for performing such baseline studies in hemodialysis centers and it is hoped that the data accumulated during the next two years in the surveillance study will assist in the study design for vaccine efficacy trials.

B. Passive Immunization

In FY73 the NIAID, in collaboration with the Veterans Administration (VA) hospitals, initiated studies to determine if high-titered anti-HB_S gamma globulin can prevent or modify HBV infection in persons experiencing accidental

parenteral exposure (Needlestick) or in persons receiving blood transfusions. While normal gamma globulin preparations have little or no effect in preventing icteric hepatitis B infection, preliminary data available suggested that gamma globulin containing anti-HB_S may be effective in preventing or modifying this disease. The studies are nearing completion and follow-up of patients is being continued. Although the code to the Needlestick study has been broken, a complete analysis of the data is required before evaluation of efficacy under these study conditions can be determined. The post-transfusion study will not yield data for hepatitis B immune globulin because the incidence of HBV infection in the groups was negligible. The use of volunteer blood and the screening of donor blood by radio-immune assay has by now virtually eliminated transfusion hepatitis due to HBV. The gamma globulin preparations for these studies were procured by the NIAID from Cutter Laboratories, Berkeley, California.

C. Epidemiology

In order to help identify "high risk" populations for further immunization trials, the IDB is supporting a Hepatitis Study Center at the West Seneca State School, Buffalo, New York. In addition to evaluating "at risk" subjects, this study is providing information on incidence, mode of transmission, and dynamics of hepatitis B in an institutionalized population.

The entire population of resident patients and health care personnel were initially tested for serum HB_SAg, anti-HB_S, and SGPT levels in a cross-sectional survey conducted in August, 1973. Subsequently, the population has been tested in four longitudinal surveys conducted in March-April, 1974, September, 1974, July-August, 1975, and February, 1976. In addition to these serologic surveys, specimens of nasal washings, urine and feces were collected from many subjects on a voluntary basis. From September 1, 1973, to December 1, 1975, approximately 147 new residents have been admitted to West Seneca Developmental Center (WSDC). All new subjects were screened initially at the time of admission. Of these, 30 subjects have been removed from the program because of return to family care, transfers from WSDC or death. Twenty-six residents were found to be anti-HB_S positive at the time of admission. The remaining 121 subjects were initially found to be seronegative for HB_SAg and anti-HB_S. All new residents are intensively surveyed every 3-4 weeks for any change in the serologic status for HB_SAg and anti-HB_S. About 200 physicians, nurses, nurses' aides and other employees of WSDC who gave written informed consent were also recruited for the hepatitis study program. These subjects are being followed in longitudinal surveys in a manner similar to the surveys being conducted in the existing residents.

The initial survey carried out in July, 1973, indicated the presence of HB_SAg in serum in nine percent, anti-HB_S in 35 percent, and the remaining 56 percent were seronegative for both. Subsequent follow-up testing conducted in March, 1974, and August, 1974, revealed that five percent and 20 percent of subjects initially seronegative for antigen or antibody developed HB_SAg and anti-HB_S, respectively. Biweekly follow-up of seronegative admissions to the school have shown six seroconversions (five percent) for HB_SAg and 29 (24 percent) seroconversions for anti-HB_S after varying intervals of institutionalization. The interval for seroconversions ranged from four months to eighteen months. Studies carried out in health care personnel show a similar risk of HBV infection as observed in the resident population.

In considering a vaccine efficacy trial in a mentally retarded population that includes children, careful attention must be given to the benefit/risk ratio. The finding that a significant proportion of these individuals had biochemical evidence of liver damage indicates that the population might benefit from a hepatitis B vaccine. The hypothetical negative consequences of such a vaccine would be weighed against positive identifiable factors prior to any vaccine evaluation.

D. Laboratory Support

The NIAID currently supports the Hepatitis Testing Laboratory at the American Red Cross. The laboratory's function is to test for hepatitis antigens and antibodies by the most sensitive techniques available. Tests available include subtype identification, radio-immuno assay procedures for antigen and antibody, and passive hemagglutination tests. During the past year, the laboratory has performed hepatitis B antigen and antibody tests for the NIAID hepatitis programs, and also for the hepatitis programs of other NIH B/I/D and the Food and Drug Administration. An additional function of the laboratory is to aid in the standardization of methods for detecting hepatitis antigens and antibodies.

Currently, one of the most sensitive techniques for the detection of HBV antigens in tissue consists of immunohistochemical assay procedures. In FY73 the NIAID established a contract for applying immunohistochemical assay techniques to the detection of hepatitis B antigens, particularly in primate specimens from animals involved in hepatitis infections. Results from these studies have contributed greatly to the concept that the DANE particle is indeed the virion of hepatitis B. By using highly specific reagents, direct and indirect immunofluorescent techniques, investigators at the Scripps Clinic and Research Foundation have shown that the core particle is produced in the nucleus of the hepatocytes and that the coat antigens are synthesized in the cytoplasma. The complete DANE particle is found in the hepatocyte cytoplasm and plasma. During the next two years, there will be a continued requirement for pathological and immunohistochemical assays of tissues from animals receiving the candidate hepatitis B vaccine.

III. Influenza

The long range goal of the Infectious Disease Branch Influenza Program is to support the development and application of knowledge which can be used to prevent or lessen the impact of future epidemics or pandemics of influenza. Areas of IDB research support are closely coordinated with other agencies of the Government (particularly BoB, CDC, and Army) in order to avoid overlap or duplication of efforts. In addition, much of the current IDB program is directly related to the efforts of the Intramural program of the Institute in the development and evaluation of an attenuated vaccine.

A. Vaccine Research and Development

During the past year, clinical studies have been conducted with a subunit vaccine (HANAFLU) prepared under contract by St. Jude Children's Hospital. The vaccine, consisting of the surface antigens of the virus (hemagglutinin and neuraminidase)

is as antigenic as the whole virus vaccine in man. When inoculated into volunteers as an aqueous preparation, the HANAFLU vaccine was shown to be essentially non-reactogenic. All data acquired thus far has been from adults -- hosts primed from past experiences with the influenza virus. The target population for the subunit vaccine is children and infants, so it is of prime importance to determine if this vaccine is non-reactogenic and antigenic in unprimed hosts. This vaccine will be tested and compared with commercially available split virus vaccines in the near future. Parameters utilized in the vaccine tests will include route of administration, dose, and effects of a booster. Efficacy data will be obtained in the event of a natural influenza outbreak.

As reported last year, an experimental inactivated influenza vaccine (monospecific for the neuraminidase surface antigen) is currently under investigation at the Children's Hospital in Buffalo. School children (ages 9-14) were vaccinated with monovalent H_3N_2 inactivated vaccine, the neuraminidase recombinant, or saline placebo (numbers = 400, 400, and 200). All preparations were essentially non-reactogenic. There was a natural outbreak of influenza following the vaccinations and attempts were made to evaluate the neuraminidase vaccine concept -- that is, permissive infection with modified illness. Although there are difficulties both in assuring that the illnesses observed were due to influenza and in documenting subclinical disease, data are being collected. Preliminary data show that serologically proven infection occurred in 44 percent of placebo vaccinees, 47 percent of neuraminidase recombinant vaccinees, and 26 percent of monovalent inactivated vaccinees. The incidence of clinical illness in the infected subjects was 80 percent in the placebo group, and 34-36 percent in the influenza vaccine groups. All the vaccinees who were infected and who showed clinical illness were found to have very low hemagglutination inhibition (HI) and anti-neuraminidase (ANAB) titers. However, vaccinees who were infected without clinical illness had 4-8 fold higher ANAB titers prior to infection than those who were infected with illness. These observations provide tentative support for the use of neuraminidase specific vaccines for protection against influenza infections, but further data are needed to better evaluate this approach. The data from this study will be exhaustively analyzed during the coming year.

Although clinical evaluations of attenuated vaccines were not performed during the past year, efforts in defining the genetic lesions and biochemical defects in several of the recombinants used for vaccine strains, were continued at Rutgers University. Because of difficulties in growing the various recombinants under the same conditions, progress in this area has been minimal. During the past two years, Case Western Reserve University has been investigating the development of an *in vitro* assay for degrees of attenuation of influenza viruses. There is a lack of information on the nature of genetic defects producing attenuation, and transfer of the temperature sensitive (ts) characteristics to other influenza variants produces recombinants of varying degrees of attenuation. Adequate levels of attenuation can only be determined in volunteers at the present time. Results accumulated over the past two years suggest that ts-recombinant strains of influenza virus can usually be ranked in order of relative virulence for man by virtue of their behavior in the ciliated epithelium of ferret trachea in organ culture. Although the most reliable indicator of relative virulence for man remains the level of temperature-sensitivity for each individual strain, the ferret organ culture assay is a good backup test to

confirm this prior to the introduction of these viruses into volunteers. In general, the ts-recombinant influenza viruses which destroy cilia and replicate to high titer at permissive and restrictive temperatures are under-attenuated for man and would not be considered as vaccine candidates for human evaluation. Those ts-recombinant influenza viruses which do not destroy cilia and replicate to low titer at permissive temperatures are over-attenuated for man and would not be considered as potential vaccine candidates for adult man. However, they may be considered for evaluation in children. Those viruses which destroy cilia and replicate to high titer at permissive temperatures, but which do not destroy cilia and either fail to replicate or replicate to low titers at restricted temperatures are usually adequately attenuated for man and deserve evaluation as potential vaccine candidates in adult volunteers.

In addition to experimental vaccines, chemotherapeutic agents are being evaluated both for prevention and therapy of influenzal disease. Amantadine is being studied therapeutically for effects on small airway passage dysfunction. It has been shown in a study at the University of Rochester that recovery of small airways following natural influenza is enhanced if amantadine therapy is employed. The mechanism of this action is currently unknown. Rimantadine, an analogue of amantadine, will be evaluated during the next contract year.

B. Immunology

Various aspects of the immunological response to live and inactivated influenza vaccines are being studied at the Baylor College of Medicine (Influenza Research Center) and the University of Rochester. There is a paucity of definitive data on protective mechanisms from influenza infection and correlates of immunity. It has been established that antibodies resulting from infection from one H_3N_2 virus can be protective against heterotypic influenza virus (H_3N_2) for at least two years. The role of cellular immunity in pathogenesis or immunity in influenza disease remains obscure. Studies with a nude mouse animal model have given results which may have implications for human disease. In athymic immunity-impaired nude mice, an influenza infection which rapidly kills furred mice leads instead to prolonged survival with late death due to encephalitis, rarely a cause of death in furred mice. This points toward some role cell mediated immunity (CMI) in the pathogenesis of this infection in mice and perhaps in fatal human infections as well.

C. Epidemiology

Numerous questions on the epidemiology of influenza remain unanswered. The Influenza Research Center at the Baylor College of Medicine is conducting a variety of surveillance studies designed to detect the earliest signal of influenza activity in a community, methods for detection of epidemics, and longitudinal family surveys. Experience with influenza in the Houston area this past winter showed quite clearly that conventional indicators of influenza activity (school and industrial absenteeism) are not sufficient for detecting the beginning of an outbreak, or even moderate levels of influenza in the community. Perhaps the earliest detection method is through culturing patients, preferably children, reporting to hospitals or clinics with febrile upper respiratory illnesses. The epidemiologic studies at Houston have been underway for one full year and data is now being analyzed from this past influenza season.

As presented in last year's annual report, the need for investigating the interactions of animal influenza viruses and human influenza viruses is quite apparent. Major antigenic shifts, characteristic of new human pandemic strains, may have resulted from the recombination of influenza viruses in some nonhuman animal species. St. Jude Children's Hospital is currently under contract to conduct intensive epidemiological and epizootiological studies of animal influenza viruses, both at a national and international level. This contract was just started this past year, but sentinel animals in the United States are being used to detect avian and swine viruses. A surveillance network is also being established through investigators and laboratories throughout the world in close collaboration with the World Health Organization. The contractor will also provide a repository for all newly isolated and characterized animal viruses and appropriate reference reagents.

D. Pathogenesis

Uncomplicated influenza has been shown to produce pathologic alterations in the tracheobronchial tree and abnormalities in pulmonary function in the absence of demonstrable changes on chest X-rays. Moreover, bacterial pneumonia is the most common and significant complication of influenza. Defense of the lower respiratory tract against microorganisms is therefore of great importance but the nature of this defense and the relative significance of different mechanisms in man are presently unknown. During the next contract year the Influenza Research Center will initiate studies of the effect of naturally occurring influenza on the development of humoral and cellular defense mechanisms in the normal tracheobronchial tree and lung, and assess measurable alterations in pulmonary function during the course of naturally occurring influenza during the next contract year.

IV. Other Viral Respiratory Infections

The goal of the IDB's Viral Vaccine Program is to develop vaccines for the prevention of the more common serious viral infections. Vaccine development studies are being performed by the scientists in the Laboratory of Infectious Diseases (LID), and by investigators supported by the IDB under contractural arrangements. Scientists at LID have prepared or are preparing mutants to influenza viruses, and respiratory syncytial virus (RSV) for use as vaccines, while investigators at Rutgers University have been developing live virus mutants to RSV and parainfluenza virus types 1, 2 and 3. Virus vaccines and virus challenge pools are prepared and safety tested by Flow Laboratories under contract to the LID.

A contract was initiated to search for evidence that all or a portion of the genome of influenza virus, RSV, or parainfluenza type 3 virus persists in the cells of the respiratory tract of man either in the form of viral RNA or as integrated complementary DNA. During this past year autopsy samples have been made available for analysis for the presence of the genome of these viruses. Testing procedures for influenza virus and RSV have been defined including establishing *in vitro* persistently infected cell cultures which serve as positive controls. Studies with parainfluenza virus type 3 are just being started. If any of these viruses is found to have the capability to persist in the respiratory tract of man, the safety of live virus vaccines will have to be re-examined.

The IDB has emphasized two kinds of vaccine evaluation centers: general study centers where the principal investigator has interest and expertise in the broad area of vaccine evaluation, and special study centers where the principal investigator has a primary interest in evaluating a particular type of vaccine. Included in our general study centers are the University of North Carolina, D. C. Children's Hospital, Baylor College of Medicine, Vanderbilt University, University of Colorado and the University of Rochester. Each of these contracts is performing studies under at least two of the vaccine program areas of the IDB.

A. Respiratory Syncytial Virus

Because RSV is one of the principal causes of severe lower respiratory tract disease among infants and children, there is a need for an effective vaccine. Scientists from LID, and from Rutgers University have obtained RSV mutants which appear genetically stable, and appear to be at the proper level of attenuation in limited in vitro studies. Viral vaccines will be prepared and evaluated in the calf and perhaps in the ferret, the animals which are being utilized as models for studies of the pathogenesis of RSV infections. A bovine respiratory syncytial virus vaccine has been prepared and is ready for evaluation in humans.

Last year the contractor at Rutgers University made the very significant discovery that RSV can enter into association with host cells in the form of a proviral DNA intermediate. He is attempting to further define the conditions necessary for this to occur. Although many investigators have cells which are persistently infected with RSV, there has been great difficulty in repeating the transfection experiments. A workshop on viral persistence and its implications was held on October 23 and 24, 1975, in conjunction with the Infectious Disease Committee meeting. Recommendations from that workshop and Committee meeting were to continue studies with live virus vaccines, and to continue looking for persistent infections. Viral persistence may be very common, may not be dangerous, and, in fact, may be one reason why immunity following natural infection or receipt of live vaccines is so long-lasting.

The ferret and the calf were selected as experimental animal models in which to study factors pertinent to disease and immunity to RSV. It has been learned that the ferret concentrates RSV-IgG three to four-fold in the immune products of lactation, as compared to the serum, and that infants nursing immune mothers are protected from challenge. Intraperitoneal or peroral administration of serum antibody does not protect the infant.

Studies in children have shown that nasal secretory antibody developed following natural infection is not neutralizing antibody. Its role is unknown. An antibody-like RSV-neutralizing substance is present in nasal secretions but its presence does not correlate with resistance to infection, nor does its level necessarily rise following infection. In a RSV-ts-1 vaccine trial at Vanderbilt University, it was shown that the presence of nasal secretory antibody at a level of 1:20 was correlated with resistance to infection with the vaccine virus. In longitudinal studies at Vanderbilt and North Carolina it has been shown that the first infection with RSV does not make the child more sensitive to the second infection . Instead, the first infection was usually the most severe with subsequent infections becoming less severe, possibly because of increasing age of the child. In contrast to influenza virus infection, RSV elicits little or no interferon in the human infant. A contractors' meeting is being planned

to discuss the latest findings on RSV, and to compare results of various serological tests.

B. Adenoviruses

Crystalline adenovirus type 5 hexon and fiber antigen vaccines were prepared at the Baylor College of Medicine. Both vaccines had been shown to be antigenic and safe in adults, young teenagers and older children. The hexon antigen, however, was not antigenic in 3-5 year old children. The vaccine either may have lost antigenicity over prolonged storage, or it may not be primary immunogen. Hexon and fiber antigen subunit vaccines from type 4 adenovirus are also being prepared and will be tested in adults to ascertain if they are immunogenic. A contractor at St. Louis University is studying both normal tissues and tumors for the presence of DNA sequences specific for adenoviruses 2, 7 and 12 to determine if viral DNA sequences can be found in tumor cells which could represent either infectious virus or integrated viral genes. This contract is funded through the BoB, FDA, with a portion contributed by the IDB because of past and possible future interest in whole adenovirus vaccines. Thus far all 98 tissues that have been analyzed for DNA sequences specific for adenovirus 12 were negative under conditions which could detect 0.2 copies of ten percent of the genome. Analysis of 91 tissues with labeled adenovirus 7 DNA and 53 tissues with labeled adenovirus 4 DNA provided no evidence for DNA sequences specific for these respiratory adenoviruses in lung tumors or normal lung tissues.

C. Parainfluenza Viruses

Parainfluenza virus type 3 is one of the principal causes of severe lower respiratory tract disease among infants and children and the need for an effective vaccine continues. Studies are continuing on 14 ts mutants of type 3 parainfluenza virus to determine their degree of attenuation, and genetic stability, while ts mutants to type 1 and 2 parainfluenza viruses are being developed at Rutgers.

During the past year studies with a live parainfluenza virus type 3 vaccine prepared by Merrell-National Laboratories were conducted at Vanderbilt. The vaccine appears to be safe; that is, it was not infective in children who were seropositive, and was infective and antigenic but did not cause disease in seronegative children. The vaccine appears to be at the appropriate level of attenuation for administration to infants but additional studies must be performed in a stepwise fashion.

D. Vaccinia Virus

During a previous study to determine the best smallpox immunization procedure, using a variety of smallpox vaccines, it was found that children who were initially immunized subcutaneously and then challenged with standard calf lymph percutaneous vaccine had only a 65% incidence of neutralizirg antibody on follow-up as compared to 88% of those who were initially immunized percutaneously. Cutaneous clinical response also differed depending on whether or not the vaccinee was immunized initially percutaneously or subcutaneously; those in the percutaneous group appeared to have better protection. Because

of this unexpected finding, a contract was awarded for the purpose of locating as many of the children as possible in St. Louis who received the subcutaneous smallpox immunizations and did not develop neutralizing antibody. Follow-up studies of these children include comparisons with children who were immunized either subcutaneously or percutaneously and developed neutralizing antibody.

V. Bacterial Diseases

A. Pneumonia

1. Pneumococcal

Through IDB contracts, monovalent and polyvalent vaccines of pneumococcal capsular polysaccharides have been developed and clinical evaluation studies on these vaccine preparations have been conducted over the past several years. Most of the work on vaccine development and the epidemiology of pneumococcal infections has been done through a contract with the University of Pennsylvania. Through a subcontract with the University of Pennsylvania, a small efficacy trial of two hexavalent vaccines has been completed in 1,300 volunteers at high risk of pneumococcal pneumonia. No adverse reactions to the vaccine were seen in the patients at Dorothea Dix Hospital, Raleigh, North Carolina.

A much larger vaccine trial is currently underway and nearing completion in San Francisco. This study, under a contract with Kaiser Foundation Research Institute, is designed to determine the efficacy of a dodecavalent pneumococcal vaccine in preventing type-specific pneumococcal pneumonia and its impact on pneumococcal pneumonia and total respiratory disease in adults over age 45.

Vaccination at Kaiser began in January and was completed at the end of December, 1974, with 13,600 volunteers receiving either a dodecavalent pneumococcal vaccine or a saline placebo. The vaccine was well tolerated by the recipients and good serum antibody responses to the individual polysaccharides have been seen. Analysis of the data from this study is currently underway. The computer data base for the Kaiser trial as well as other pneumococcal pneumonia vaccine studies is maintained by Dr. Austrian in Philadelphia.

An octavalent pneumococcal polysaccharide vaccine was evaluated for antigenicity and toxicity in infants and children under a contract with the University of California at San Francisco which was terminated during the past year. The studies have shown that the vaccines are non-reactogenic and give good antibody response in children over two years of age, although infants a year old or younger respond poorly to the pneumococcal antigens. This study has also provided an indication of the value of the pneumococcal vaccine for preventing pneumococcal disease in splenectomized children and children with sickle cell disease, a group particularly disposed to pneumococcal infections. Although this contract was terminated due to the investigator's difficulty in obtaining volunteers (due to restrictions placed on volunteer studies), the Branch continues to collaborate with investigators interested in studying preumococcal polysaccharides in splenectomized and sickle cell children by supplying vaccine and performing serologic assays through its reference laboratory contractor at SUNY-Downstate Medical Center.

2. Mycoplasma

A formalin-inactivated Mycoplasma pneumoniae vaccine (designated OSU-1A) was produced under a now expired contract with Ohio State University. The vaccine was tested for safety and potency at Ohio State and at Cornell University.

A contract was awarded in FY74 to the University of Virginia to study the clinical acceptability, antibody response, and efficacy of the OSU-1A vaccine. Following small scale toxicity and antigenicity studies at Virginia, a field trial was performed at the Parris Island Marine Corps Recruit Camp in South Carolina. Military recruits, particularly in this geographical area, are at high risk to Mycoplasma pneumoniae. From July to September, 1974, 7,861 volunteers received either the OSU-1A vaccine or a saline placebo. The subjects were followed for disease incidence and antibody response through their eight weeks training period at Parris Island and approximately ten percent to twenty percent (those assigned to Camp Lejeune, North Carolina, after basic training) were followed for an additional year.

Analysis of the data available to date shows that the vaccine is non-reactogenic and safe in a military group at high risk of acquiring M. pneumoniae illness. The vaccine has also shown efficacy with a 52 percent reduction in total pneumonias and a 67 percent reduction of M. pneumoniae specific (as determined by isolation of the organism) pneumonia. A thorough analysis of seroconversions in specimens from the Parris Island trial is nearing completion at Virginia and in the laboratory of the Branch contractor at Cornell University. This data will be combined with that on isolation and clinical disease to give the final figure on vaccine efficacy. It is expected that this study will be completed during the next contract year.

Under a contract with Baylor College of Medicine, studies are being conducted to develop an experimental challenge system for M. pneumoniae infections. Large pools of broth and tissue culture grown M. pneumoniae have been prepared and stored. They will be used for experimental infection studies in humans by intranasal and small particle aerosol inoculation for evaluation of other candidate vaccines such as attenuated Mycoplasma. It is anticipated that all the studies on the inactivated M. pneumoniae vaccine will be satisfactorily completed and phased out during the coming year.

B. Meningitis

Bacterial meningitis (excluding tuberculous meningitis) accounted for approximately 2,000 reported deaths in the United States per year from 1970 through 1973. This number suggests an annual occurrence of 20,000. Two organisms (Hemophilus influenzae type b and Neisseria meningitidis) are primarily responsible for bacterial meningitis in young children and infants. In the age group two months to five years, H. influenzae causes as much as eighty percent of the cases while pneumococcus and meningococcus cause most of the remainder. Between the ages five and nineteen (inclusive) N. meningitidis is the predominant pathogen. The meningitis vaccine program of the Infectious Disease Branch is directed largely toward the pediatric population and is therefore concerned with these two causal organisms of meningitis.

A candidate H. influenzae type b vaccine consisting of purified polyribose phosphate (PRP) capsular material was developed at the Children's Hospital Medical Center in Boston. This group, as well as contractors at the University of Southern California, the University of Colorado, and the University of Rochester have been performing clinical evaluation studies of PRP in children for the past several years. Children's Hospital, Boston, serves as a serological reference laboratory performing radio-immuno assays for anti-PRP antibodies in most sera obtained in these projects. The results from all these investigations on PRP indicate that the purified polysaccharide is immunogenic in children over the age of 24 months but that there is very little antibody response in infants under 18 months of age. The H. influenzae PRP was used as a control vaccine in a field trial of the meningococcal A polysaccharide vaccine in Finland (described below) and preliminary data from this study appear to show a protective effect for this vaccine against meningitis in children over two years of age and a general lack of effect in infants.

As H. influenzae disease is the major cause of meningitis in infants, the Branch program is now being directed toward the development of alternate antigen preparations which may prove more immunogenic than PRP in this age group. The contractor at Boston Children's Hospital has developed a new candidate H. influenzae vaccine which is prepared via a variation in the purification procedure used for PRP. The resulting product is a complex of PRP, five or more polypeptides, and a trace of lipopolysaccharide. The new candidate material, called PRPc, has, unlike the purified PRP, been shown to be highly immunogenic in weanling rabbits. It, hopefully, will also prove to be more immunogenic in infants. The preparation has been tested in a limited number of adults and appears to be a good immunogen; however, some adverse reactions have been observed. Efforts are currently directed toward reducing the toxicity of the preparation. If the toxicity can be reduced and immunogenicity maintained as judged by human adult studies, further clinical evaluation in infants will be carried out by IDB meningitis vaccine contractors.

Clinical studies in children of the groups A and C meningococcal polysaccharide vaccines developed by the Walter Reed Army Institute of Research are being carried out by contractors at the University of Connecticut, the University of Colorado, the University of Rochester and the University of Southern California. A contract with Rockefeller University supports a reference testing laboratory to measure, via radio-immuno assay, the antibody response in sera from infants and children immunized with meningococcal polysaccharide vaccines.

Group A and Group C meningococcal polysaccharide vaccines have been licensed for use in adults; however, they have not yet been licensed for routine use in young children and infants where a large percentage of the disease occurs. For this reason, the efforts of the Infectious Disease Branch continue to be directed toward vaccines for the pediatric population.

In general, results from clinical studies indicate that the quantitative ability of infants to produce antibodies to the meningococcal groups A and C polysaccharides is a function of the age of the infant, vaccine dose, molecular weight of vaccine, prior immunization with vaccine, and prior exposure to naturally occurring cross-reactive antigens. The Connecticut contractor has shown that good antibody responses can be elicited in very young infants with

meningococcal group A vaccine using a two dose regimen. The response of infants to group C vaccine, however, is not increased with boostering. Primary immunization at an early age (i.e., three months) appears to cause a dampening of the response to group C vaccine when given later (i.e., 12 months of age).

Because of the observation of the "dampening" of the group C vaccine when three-month-old infants receive the vaccine, no new clinical studies are being done with the group C vaccine in this age group. The Branch has recently started clinical studies of another group C polysaccharide vaccine. This material is extracted from a variant strain of group C meningococcus and has a slightly different molecular structure. It will be evaluated for safety and antigenicity in adults at the University of Rochester prior to initiation of clinical studies in children.

In 1974, a contract was awarded to the Central Public Health Laboratory, Helsinki, Finland, to conduct an efficacy trial of the group A meningococcal polysaccharide vaccine in children ages three months to six years. The project was initiated in response to request by representatives of the Laboratory and the Finnish National Board of Health. At that time, Finland was in the midst of a moderate epidemic of group A meningococcal disease. The meningococcal vaccine was supplied by Merck, Sharp and Dohme. H. influenzae PRP, prepared under the IDB contract with Boston Children's Hospital, was selected as a control vaccine because of the high incidence of H. influenzae meningitis in Finland.

Preliminary results from the Finnish trial, which involved 100,000 children in three provinces of the country, indicate that group A meningococcal vaccine was successful in protecting against group A disease throughout the entire age group. As mentioned previously, the H. influenzae vaccine appears to protect over 18 months of age but to be ineffective in younger children. The contract may be continued for another year for additional follow-up, particularly with reference to H. influenzae disease.

Several laboratories are working on protein antigen preparations which may serve as potential candidates for a group B meningococcal vaccine (the group B meningococcal polysaccharide is non-antigenic in humans). One such protein preparation, from the outer membrane layer of the organism, is in the animal safety and immunogenicity testing stage at the Bureau of Biologics. Tentative plans are to do safety and antigenicity studies in adults in the fall of 1976. If these adult experiments warrant further investigation, clinical evaluation will be extended to studies in children.

In order to explore the possibility of conducting a field trial of meningitis vaccines in the United States, the contractor at the University of Southern California was asked to develop a system for surveillance of meningitis cases in Los Angeles County. As of the end of 1975, 146 out of 194 hospitals in Los Angeles County were enrolled in the study. An analysis of the number of cases with regard to their census tract and hospital location is in process at the present time. Continuation of the study past summer of 1976 will depend upon the incidence of H. influenzae and meningococcal disease seen in the area during the past year of surveillance.

C. Otitis Media

Acute otitis media is a very important childhood disease and affects populations of all racial and social classes. Almost every child has otitis media at least once and 20 to 30 percent have six or more such occurrences. It is well established that pneumococci are responsible for 40 to 50 percent of the episodes of acute otitis media.

Acting on the recommendation of the Infectious Disease Committee and a Branch sponsored workshop in December, 1974, contracts were awarded to study the value of the pneumococcal polysaccharide vaccines in preventing pneumococcal otitis media in infants.

Contracts were awarded to Boston City Hospital and Huntsville Hospital (Alabama) to conduct trials of the efficacy of an octavalent vaccine in preventing pneumococcal otitis media in infants and young children who have had previous middle ear infections. In each of these studies, 200 or more children will receive either the octavalent vaccine containing serotypes commonly causing otitis media or a control vaccine containing seven polysaccharides of other pneumococcal types less important in middle ear disease. During the first year, both of these contractors have been recruiting volunteers into their study and have begun vaccinations. Both studies are proceeding on schedule. No data on serologic response or efficacy are available at this time.

Under a contract with Vanderbilt University, further studies toward determination of the optimum dose and booster regimen for maximum antibody response of infants and young children to the octavalent pneumococcal polysaccharide vaccine are underway. This is a continuation of work initially done at the University of California and is extending earlier investigations to a larger number of children with a more varied dose and booster regimen. This study is proceeding on schedule with 51 volunteers enrolled at the present time.

In order to study the pathogenesis and immunology of pneumococcal otitis media and the efficacy of pneumococcal polysaccharide vaccines in preventing experimentally induced middle ear disease, a contract was awarded to the University of Minnesota in FY75 for development of an animal model. The contractor, during the first year, has made progress in developing this model in chinchillas; infections with type 23 and type 3 pneumococci have been achieved in the middle ear and the immunopathologic responses have been shown to vary greatly. Further studies with other pneumococcal serotypes and protection studies with the pneumococcal polysaccharides are underway.

A contract with the State University of New York - Downstate Medical Center supports a serologic reference laboratory for the total IDB pneumococcal vaccine program. The contractor measures, via the radio-immuno assay, all sera from the otitis media contractors for antibody to pneumococcal polysaccharides and also supplies radioactively labeled polysaccharides to the other IDB contractors who perform their own radio-immuno assays.

D. Streptococcal Infections

Group A streptococcal infections play a major part in septic pharyngitis and skin infections as well as an antecedent role in rheumatic fever and acute glomerulonephritis.

A contract with the University of Chicago has been in operation for several years to develop vaccines for group A streptococci. Several candidate M-protein antigens were developed and evaluated for safety and immunogenicity in adult volunteers. The objective of this study has been to determine whether a vaccine composed of M-protein is efficacious against disease.

Although it appears that type-specific protection can be conferred with M-proteins, the inability to produce highly purified antigens and the number of serologic types which would be required in a candidate vaccine have led to a planned phaseout of this contract activity.

A new program in the area of group B streptococcal infections has been proposed to the Institute and, hopefully, positive steps will be made in this direction during the next year. Group B streptococci are a major cause of neonatal infections and death and the interest of members of the Infectious Disease Committee and recommendations from recent NIH workshops in this area have stimulated efforts toward development of this new effort.

VI. Antiviral Substances Program

The treatment of established viral infections is limited to supportive measures which provide at best symptomatic relief since little can be done to alter the course of the virus infection itself. The only measures available for defense against viral infections are prophylactic, such as gamma or immune serum globulins or vaccines when available. There are many viral diseases which are serious public health problems for which such measures are either impractical or unavailable. Still others exist for which the available immune prophylaxis is only partially effective. There is, thus, a need to develop antiviral substances which can serve either as therapeutic or prophylactic agents for a broad variety of viral diseases.

Planning for a program to help meet this need began in July, 1969. At that time a thorough study of the field revealed considerable and growing interest in this area and interferon or inducers of interferon were considered the most promising antivirals to exploit. Since the interferons represent the best hope for the development of a broad spectrum antiviral agent, interest in this substance remains high. There is also a growing interest in other chemotherapeutic agents such as adenine arabinoside (ara-A), phosphonoacetic acid (PAA), trifluorothymidine (F_3T), and idoxuridine (IDU). The program continues to be alert to new developments emanating from scientist-originated basic research in order to provide the needed stimulus to bring this knowledge into practical use in man. The intent of the program is to serve as a focus for all activities on antivirals conducted by Extramural, Intramural and Collaborative Research of the National Institute of Allergy and Infectious Diseases and to some extent other Institutes of the National Institutes of Health.

Following the advice of advisory groups including the Infectious Disease Committee, there has been a shift in emphasis toward more clinical studies as agents are developed to the point of clinical testing. Clinical testing under contract support is being conducted with interferon and ara-A and trials involving PAA and adenine arabinoside 5'monophosphate (ara-AMP) are scheduled to begin during 1976.

A. Nonclinical Research and Development

Standards and Reagents: In collaboration with the Research Resources Branch, the Antiviral Substances Program (ASP) has successfully established reference reagents for rabbit interferon, mouse interferon, and interferon inducers [polyinosinic·polycytidylic acid (poly I·poly C)]. The ASP has developed methodologies for stabilizing lyophilized rabbit, mouse and human leucocyte interferon and is currently developing a reference reagent for human fibroblast interferon through a contract with the Medical College of Wisconsin. These reagents have undergone standardization analyses with unitage assigned which properly corresponds to the original materials established as standards. A contract, now terminated, produced specific antisera against human interferon which contained antibodies to some non-specific protein materials, as well as interferon. A contract is currently in progress with the Medical College of Pennsylvania which utilizes newly developed techniques to obtain monospecific antibody. The method is studying the use of mutagenized mouse lymphocytes as a means of producing highly specific interferon antibody. This will permit in turn the development of more highly purified interferon through the use of affinity chromatography.

Purification: Studies on purification of human interferon are currently being conducted through a collaborative effort involving NIAMDD and the intramural and collaborative components of NIAID. The ultimate goal is to develop a highly purified interferon in sufficient amounts to permit the definition of its chemical structure.

The use of immunoaffinity chromatography techniques with specific interferon antisera has been the most effective approach in the preparation of purified interferon. Purified interferon is much less stable than the impure form and as progress is made in its purification, it becomes important to develop techniques for stabilization of this material. A contract with the Medical College of Wisconsin is now in its second year to determine the most effective way of maintaining the stability of highly purified interferon. The studies to date indicate that purified human fibroblast interferon can be effectively stabilized against thermal inactivation at 68°C by reducing the pH to 2 or making the solution 2-molar in sodium thiocyanate or guanidine thiocyanate. Crude interferon is very stable to mechanical or vortical agitation, but purified interferon will lose 90% of its activity during one minute of vortical agitation. As little as 0.001% Tween-80 protects interferon against vortical inactivation.

Production - Exogenous and Endogenous: Two methods of administration are available for therapeutic trials of interferon in human viral diseases: the use of exogenous human interferon, produced *in vitro*, and endogenous interferon, produced *in vivo*, through the use of inducers.

For the use of exogenous human interferon, there is a need to develop techniques to provide more efficient, safe, and economically feasible methods to produce large quantities of human interferon at a reasonable cost. To this end, two contracts are currently being supported, one to develop better production methodologies including superinduction (New York University) and the other contract is testing the design and hardware of methods of large-scale production of human interferon (Lee-Mac). The work at NYU involves development of new superinduction techniques that would produce higher interferon yields, attempts to increase interferon yields by "positive transcriptional stimulation" with steroids and cyclic nucleotides, attempts to isolage high-producing clones of FS-4 cells after transformation with SV-40, and production and delivery of no less than 100 million reference units of concentrated human interferon.

The project to develop large cell cultures for interferon production has progressed well. The work with combinations of cycloheximide and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) appears particularly promising but the remaining problem is to reach effectively all the cells with the inducer. A design employing minature cell chambers has been developed so that a variety of techniques to accomplish this goal can be tested. The ASP is also collaborating with intramural staff on the development of a new and promising interferon inducer (poly I·poly C·poly l-lysine complex). This substance is currently undergoing evaluation as a topical antiviral in herpes keratitis and is being administered investigationally to cases of herpes encephalitis.

Animal Models: A critical part of the effort to develop antivirals with a potential for clinical use has been the establishment of a series of animal-viral disease models which closely mimic viral diseases of man. This constitutes the Cooperative Antiviral Testing Group of the ASP. The models selected include a wide range of animal species and viruses including nonhuman primates. Through the use of these models, comparative in vivo evaluation of antivirals is prophylactic and/or therapeutic agents is possible. In these models the route and dose of infection have been chosen to coincide as closely as possible with those of the natural infection.

The standard reference used to compare in vivo efficacy of these compounds is exogenous and endogenous interferon. When the compounds are shown to be efficacious and non-toxic in these systems, they are considered as candidates for clinical use in humans. Examples of compounds tested by this method and found to have clinical potential are ara-A, ara-AMP, and PAA. Animal models currently in use in this system include the mouse (NYU and the University of Utah), the rabbit (NYU), the pig (the University of Wisconsin), the cat (Cornell) and the squirrel and patas monkeys (Delta Primate Center, Tulane). More than a dozen virus strains, including both DNA and RNA viruses, are utilized with the models. It is hoped that the use of this system will greatly facilitate the rapid evaluation of newly identified antivirals for their clinical potential. Once this has been established, clinical studies can be considered.

B. Clinical Evaluation

Clinical Studies: Clinical trials are currently underway with ara-A in a multicenter study of the compound's efficacy in serious herpesvirus infections. Specifically, it is being evaluated in herpes encephalitis, early disseminating herpes zoster, neonatal herpes and other serious herpesvirus hominis infections, particularly in the immunologically compromised patients. Ara-A has demonstrated efficacy in the disseminated zoster study with highly significant reductions seen in new vesicle formation, vesicle virus titers and resolution time of the cutaneous disease in patients treated with ara-A as compared to the placebo group. The cross-over design of the study wherein all patients were changed over to the alternate therapy after five days prevented an assessment of the drug's efficacy in preventing visceral disease or late sequelae. A straight placebo-controlled double-blind study is now in progress to determine this. The multicenter contract is being conducted through a primary contract with the University of Alabama.

Exogenous human leucocyte interferon is currently being tested under contract for efficacy against both zoster and varicella infections in patients with malignancy in a double-blind, placebo-controlled study at Stanford University. In 55 of these patients who entered the study early in the course of their infection, a five-fold lesser frequency of visceral complications was observed in the interferon treated group. The group also showed a reduction in pain in the primary dermatome. Other contract studies currently underway involve clinical trials of exogenous interferon in ocular herpes and cytomegalovirus infections, but definitive results are not yet available.

A clinical study utilizing exogenous interferon for the eradication of the chronic hepatitis B carrier state is also underway. Patients will be treated for extended periods with the hope that the viral production can be aborted.

Since exogenous interferon in huge doses was shown to have efficacy against rhinovirus infections and possibly influenza, a small contract at Baylor University has been developed to determine why such unexpectedly large doses of interferon are required for effect. The study includes a search for inhibitors in the nasal secretions plus a study of the sensitivity of nasal mucosal cells to the protective effect of interferon. Preliminary results suggest that the mucosal cells are relatively insensitive to interferon and that the secretions may contain an inhibitor to fibroblast, but not to leucocyte interferon.

A large scale double-blind, placebo-controlled efficacy study of PAA and ara-AMP against herpes labialis will be inaugurated under contracts in Boston, Miami and Salt Lake City. A preliminary study of the natural history of herpes labialis under these three contracts has demonstrated the practicality of utilizing this form of herpesvirus hominis (HVH) infection as a measure of drug efficacy. If the current studies of ara-A in serious herpesvirus infections continue to show efficacy, ethical considerations will probably prevent testing other new promising antivirals against these infections. Herpes labialis thus appears to be a useful and simple alternative as a human model for the future.

Individual Contract and Agreement Tabulation

PROGRAM: SEXUALLY TRANSMITTED DISEASES - GONORRHOEAE AND SYPHILIS

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Brinton, Charles C. AI 52539 University of Pittsburgh $67,000 (for six months)	To develop a vaccine for the prevention of gonorrhea using purified gonococcal pili as the candidate immunizing material. To develop and test the usefulness of a serologic typing system for the gonococcus based on the serologic differences in gonococcal pili. The contractor has further developed the procedures for production and purification of large quantities of gonococcal pili. Twelve gonococcal "auxo-serotypes" have been established by a combination of serotyping and auxotyping. Chimpanzee experiments for the protective effect of a pilus vaccine have yielded data which indicate protection but are not conclusive. Further animal and human studies will await completion of definitive work on the chemical nature of the pili and their serologic determinants.
Buchanan, Thomas M. AI 52535 University of Washington $0 (multiple year funded in FY75)	To conduct studies toward the development of a serologic typing system for Neisseria gonorrhoeae. The contractor is studying principal outer membrane protein and pili as candidate antigens on which to base a serotyping system. Progress has been made with both approaches. Antigenic heterogeniety of gonococcal pili has been shown using direct hemagglutination, direct agglutination and Ochterlony immunodiffusion techniques. The radio-immuno assay appears to be less specific. Changes in chromatographic techniques and use of 2-dimensional immunoelectrophoresis have resulted in an improved procedure for purification of the principal outer membrane protein for use in developing serotyping antisera.

PROGRAM: SEXUALLY TRANSMITTED DISEASES - GONORRHOEAE AND SYPHILIS

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Fieldsteel, A. Howard AI 42536 Stanford Research Institute $0 (funded in in FY74)	To develop methods for the *in vitro* cultivation of *Treponema pallidum*, the causal organism of syphilis. The contractor has developed the facility of working with the treponemes and has studied various tissue culture systems to determine the optimal cell and media conditions for maximum maintenance of motility. Data obtained from this study has added additional evidence to the current hypothesis that *T. pallidum* is microaerophilic rather than anaerobic. Methods are being developed for the isolation of radioactively labeled DNA from various treponemes for use in hybridization experiments. These experiments will be designed to show the genetic relationship between *T. pallidum* and the cultivable treponemes.
Jenkin, Howard M. AI 42537 University of Minnesota (Hormel Institute) $79,000	To develop methods for the *in vitro* cultivation of *Treponema pallidum*, the causal organism of syphilis. This contractor has made important advances in studies of co-cultivation in tissue cultures and the use of "spent" tissue culture medium for providing proper environment for maintenance of treponeme motility. Human foreskin tissue culture has been shown to be most successful in maintaining viability in these studies.
Johnston, Kenneth H. AI 52534 University of Texas Health Science Center, Dallas $29,000	To develop a serologic typing system for *Neisseria gonorrhoeae* using the major outer membrane protein as the basic antigen of the system. Using this protein antigen, the investigator has established several distinct serotypes. During the past year, an additional serotype has been added to the scheme giving a total of sixteen. Studies will continue to further define the chemical nature of the antigen, to examine additional strains with the serotyping scheme, and to determine the relationship between serotype and auxotype.

PROGRAM: SEXUALLY TRANSMITTED DISEASES - GONORRHOEAE AND SYPHILIS

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Miller, James N. AI 42538 University of California at Los Angeles $73,000	To develop methods for the *in vitro* cultivation of *Treponema pallidum*, the causal organism of syphilis. The contractor has made further progress toward defining the optimal tissue culture medium and environmental conditions of incubation for maintenance of viability of *T. pallidum*. Motility has been shown to be prolonged by peroxidase under aerobic conditions, but not affected by other antioxidants under the same conditions. They have also found that fresh normal rabbit serum is superior to fetal calf serum as an additive to basal medium for maintenance of motility of treponemes. Progress has been made toward defining the best conditions for purification of the organism in density gradient systems.

PROGRAM: HEPATITIS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Chisari, Frank AI 32509 Scripps Clinic and Research Foundation $25,000	To perform immunohistochemical assays of biopsy samples for hepatitis virus associated antigens. Studies in refinement of techniques and immunohistochemical assays. Currently assaying biopsy samples from chimpanzees on hepatitis B vaccine tests.
Coulston, Fred IA 30002 Albany Medical College $81,000	To establish and maintain a breeding colony of chimpanzees. Collaborative effort of NIAID, FDA, and CDC for ensuring chimpanzees for experiments with viruses of hepatitis. Program is effective and approximately 25 newborns are being produced annually.
Gerone, Peter J. AI 12197 Tulane University $13,000	To provide a facility for studying hepatitis B infections in chimpanzees. Animals have been inoculated with hepatitis B vaccine material and will be on test for another two years. A total of four animals can be handled in isolation rooms.
Gerone, Peter J. IA 40005 Tulane University $0	To establish and maintain a breeding colony of Saguinus mystax marmosets. Collaborative effort of NIAID, FDA, and CDC for ensuring future availability of small animal model for hepatitis A experiments. Fourteen animals were captured and placed in colony last year. Field team planning to return to Brazil to capture more breeding stock. One birth in breeding colony last year.
Nath, Nrapendra AI 22522 American Red Cross $299,000	To provide a Hepatitis Testing Laboratory to perform assays for hepatitis B antigens and antibodies. In addition, the testing laboratory is involved in developing better methods for detection and testing of HBV antigens and antibodies. The laboratory provides a testing for other ongoing NIAID hepatitis projects, as well as services to other NIH research projects.

PROGRAM: HEPATITIS

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Ogra, Pearay AI 32522 State University of New York (Buffalo) $124,000	To perform an epidemiologic survey of hepatitis B in a population in which viral hepatitis is highly endemic. The study encompasses the descriptive epidemiology of hepatitis B in both patients and staff and is carefully designed to document the incidence and prevalence of HB_SAg infection and illness, and the development of anti-HB_S.
Prince, Alfred AI 62500 Lindsley F. Kimball Research Institute of The New York Blood Center $114,000	To test a prototype bivalent hepatitis B vaccine, developed by the NIAID, in chimpanzees. Approximately 50 seronegative chimpanzees located at the Liberian Biomedical Research Institute will be used for determining safety, antigenicity, and efficacy of the vaccine. The animals are out of quarantine and testing will begin in June, 1976.
Rogers, Leyton M. AI 32504 Meloy Laboratories $235,000	To maintain an animal holding facility for hepatitis infected chimpanzees and other non-human primates. Hepatitis B vaccine safety, antigenicity, and efficacy in chimpanzees under test at this facility. Essentially a service contract.
Seeff, Leonard IA 20002 Veterans Administration $62,000	To conduct two double-blind studies administering (I.M.) either conventional low titer or high titer anti-HB_S gamma globulin to determine if these preparations lower the incidence of hepatitis B infections in certain "high risk" populations. Studies are in final phase of following participants and data are being analyzed. One study, the post-transfusion study, will not contribute to the evaluation of immune globulin because the attack rate of hepatitis B was negligible.

PROGRAM: INFLUENZA BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Oblications	Objectives and Findings
Couch, Robert B. AI 42528 Baylor College of Medicine $0 (Previous Year's Funding)	To establish and maintain an Influenza Research Center. The Center is conducting comprehensive studies of pathogenic, epidemiologic, and immunologic aspects of influenza, as well as evaluating methods of disease prevention and/or control. Included are testing of licensed vaccines, experimental vaccines (live and inactivated), and potential antiviral substances. A variety of surveillance studies for detecting the earliest signal of influenza in the community, and longitudinal family studies are underway. The Houston Area experienced an outbreak of influenza (A/Victoria) this past winter and the accumulated data are being analyzed. A number of vaccine trials (licensed and subunit) were conducted during the past year. It was shown that a subunit vaccine under development by the NIAID was as antigenic, but less reactogenic, than whole virus in adult volunteers.
Denny, Floyd W., Jr. AI 22506 University of North Carolina $31,000	To maintain a surveillance program for influenza virus activity in selected populations. The surveillance includes appropriate serum specimens and materials for virus isolations from suspected cases of influenza. In the event influenza is detected, attempts will be made to identify the A/Influenza isolates.
Douglas, R. Gordon, Jr. AI 22503 University of Rochester $74,000 (40%)	To clinically evaluate viral vaccines as they become available (live and inactivated influenza, live RSV, and live parainfluenza viruses). The major emphasis is on the evalution of influenza virus vaccines for infectivity, antigenicity, acceptability, and transmissibility in normal adults and people at high risk of mortality from influenza; for example, the elderly. During the past year, in the absence of vaccines to be tested, studies of peripheral airway dysfunction in patients with influenza and the influence of administration of Amantadine on this abnormality, and the shedding of influenza virus by children, were conducted. See also Other Respiratory Infections (35%) and Bacterial Diseases (25%).

PROGRAM: INFLUENZA BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Gwaltney, Jack M., Jr. AI 22508 University of Virginia $21,000	To initiate and maintain a surveillance program for influenza virus activity in selected populations. This surveillance includes screening a set number of adults and children with febrile respiratory disease one day each week, serological facilities to obtain baseline information and to monitor individuals 25 years and younger for influenza activity.
McIntosh, Kenneth AI 22501 University of Colorado $24,000 (30%)	To initiate and maintain a surveillance program for influenza virus activity in selected populations. This surveillance includes screening a set number of adults and children with febrile respiratory disease one day each week, serological facilities to obtain baseline information and to monitor individuals 25 years and younger for influenza activity, and maintaining the capacity to respond to outbreaks of influenza in Colorado with both culture and serologic capability. See also Other Respiratory Infections (30%) and Bacterial Diseases (40%).
Mostow, Steven R. AI 42542 Case Western Reserve University School of Medicine $0	To develop an *in vitro* assay for attenuation of live influenza viruses. Results suggest that ts recombinant strains of influenza virus can usually be ranked in order of relative virulence for man by virtue of their behavior in the ciliated epithelium of ferret trachea in organ culture. The most reliable indicator of relative virulence for man remains the level of temperature-sensitivity of each individual strain. The ferret organ culture assay is a good back-up test to confirm this level of temperature-sensitivity prior to the introduction of these viruses into volunteers. Terminating - principal investigator relocating.

PROGRAM: INFLUENZA BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Ogra, Pearay AI 42511 State University of New York (Buffalo) $0 (Previous year funding)	To conduct a clinical field trial with X41 (H Port Chalmers/N Port Chalmers), X42 (H equi 1/N Port Chalmers) inactivated influenza virus vaccines and placebo. Approximately 1,000 school children (ages 9-14) received vaccines and placebo. The vaccines were antigenic and essentially non-reactogenic. Buffalo experienced an outbreak of A/Port Chalmers influenza the winter following vaccination. Data is still being accumulated and analyzed for determining the efficacy of the vaccines.
Parrott, Robert H. AI 12091 Children's Hospital of D.C. $80,000 (50%)	To evaluate candidate viral vaccines for immunogenicity and safety in infants and children. Studies of factors important to the pathogenesis of viral diseases or which affect the host's resistance to infection. Safety and antigenicity testing of live influenza vaccines in children is underway. Long-term surveillance of vaccinees continues. See also Other Viral Respiratory Infections (50%). Intramurally funded Gastroenteritis ($68,000).
Simpson, Robert W. AI 42509 Rutgers University $0	To evaluate influenza temperature sensitive mutants for phenotype, genetic defect, and infectious potential. Contract terminated.
Webster, Robert G. AI 42510 St. Jude Children's Hospital $55,000	To isolate and purify various polypeptide antigens of influenza virus for evaluation as subunit vaccine. Results of several clinical trials with adult volunteers indicate that subunit vaccine is as antigenic as whole virus vaccine, but not as reactogenic. Vaccine preparation consisted of purified hemagglutinin and neuraminidase subunits.

PROGRAM: INFLUENZA

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Webster, Robert G. AI 52524 St. Jude Children's Hospital $0 (Previous year funding)	To establish a reference bank of animal influenza viruses. Efforts are directed to organizing an international network for the surveillance of influenza infections among domestic and wild species of animals. Viruses isolated will be placed in the repository and should be manipulated in a manner to ensure accurate passage histories and maximum growth in materials suitable for production of vaccines in man. This contract was started last year, so most efforts have been devoted to organizing the surveillance networks.
Wright, Peter F. AI 22507 Vanderbilt University $53,000 (25%)	To maintain a center for the clinical evaluation of live virus vaccines with major emphasis on parainfluenza, live respiratory syncytial and influenza virus vaccines in a semiclosed population of infants (more than one year old), and young children. Parameters studied include infectivity, degree of virulence, and production of antibody produced by these vaccines. Contractor is prepared to measure parameters in carefully monitored open populations of children and/or infants. See also Other Viral Respiratory Infections (75%)

PROGRAM: OTHER VIRAL RESPIRATORY INFECTIONS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Cherry, James D. AI 52523 University of California, Los Angeles $5,000 (overhead adjustment) (Funded for 15 months from FY75. Plus increased overhead from FY76.)	To study various parameters of immune response in children who received smallpox immunizations subcutaneously followed by percutaneous smallpox immunizations which did not result in a "take" in the vaccine recipient. Results are not yet available.
Couch, Robert B. AI 32506 Baylor College of Medicine $195,000 (70%)	To evaluate candidate vaccines for safety and efficacy in closed and open populations of adult volunteers and study various parameters of specific viral diseases. Among such vaccines was adenovirus type 5 hexon antigen which was found not reactogenic in children 3-5 years of age, but only 2/7 had an antibody response. Adenovirus type 4 subunit vaccines are being prepared for evaluation first in animals and then in adults. Studies of lymphocytes in the peripheral blood have been done during the incubation period, and illness of influenza and rhinoviruses. Laboratory studies and surveillance of respiratory viruses in various populations is on-going. See also Bacterial Diseases (15%) and Antiviral Substances (15%).
Douglas, R. Gordon, Jr. AI 22503 University of Rochester $64,000 (35%)	To clinically evaluate viral vaccines as they become available. Surveillance has shown that viral respiratory agents are important causes of nosocomial infections in the pediatric age group. Natural RSV infections stimulate negligible amounts of interferon - in contrast to influenza virus infections. See also Influenza (40%) and Bacterial Diseases (25%).

PROGRAM: OTHER VIRAL RESPIRATORY INFECTIONS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Green, Maurice IA 50003 St. Louis University $10,000	To analyze human cancers for copies of gene sequences specific for human adenovirus types 2, 7 and 12. Highly specific and sensitive nucleic acid probes have been developed which will detect extremely low levels of viral genetic material. No evidence for DNA sequences specific for these adenoviruses has been found.
McIntosh, Kenneth AI 22501 University of Colorado $24,000 (30%)	To conduct laboratory and clinical studies to evaluate efficacy of viral and bacterial vaccines. Analysis of data obtained in 70 children with RSV infections will be performed. A non-neutralizing IgA in convalescent secretions will be studied further. A neutralizing activity in secretions, which does not correlate to resistance to infection, and does not rise following infections with RSV is also being studied. See also Bacterial Diseases (40%) and Influenza (30%).
Parrott, Robert H. AI 12091 Children's Hospital of D.C. $80,000 (50%)	To evaluate candidate viral vaccines for immunogenicity and safety in infants and children. To study factors which are important in the pathogenesis of viral diseases or which affect the host's resistance to infection. Vaccine testing and long-term surveillance of vaccinees continues. See also Influenza (50%) and Intramurally funded Gastroenteritis ($68,000).
Pons, Marcel W. AI 42543 Public Health Research Institute of the City of New York, Inc. $53,000	To search for evidence that all or a portion of the genome of influenza virus, RSV or parainfluenza type 3 virus persists in the cells of the respiratory tract of man either in the form of viral RNA or as complementary DNA. Nucleic acid probes and a persistently infected cell culture of RSV are available and testing of respiratory tract samples has been initiated.

PROGRAM: OTHER VIRAL RESPIRATORY INFECTIONS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Porter, David D. AI 52517 University of California, Los Angeles $55,000	To define and study factors important in the development of RSV bronchiolitis or pneumonia in an infant animal model, the ferret. To study the role of immunologic factors in resistance to RSV infection and disease. To evaluate vaccines and/or antiviral substances in the model. RSV A_2 Long strain is being passed serially in ferrets to increase virulence in the ferret. Passively acquired antibody obtained from the "immune products of lactation" protects the infant from infection whereas serum antibody administered perorally or intraperitoneally does not. Antibody obtained by nursing appears to be transferred intact to the blood.
Simpson, Robert W. AI 22504 Rutgers University $109,000	To develop attenuated mutants of RSV and parainfluenza viruses as potential vaccines. Efforts have been directed at describing the proviral DNA intermediate of RSV in HEp-2 and BEK cell, as well as demonstrating viral persistence in ferrets, hamsters, and nude mice. Reproduction of infectious DNA in cells infected with RNA virus (RSV) is difficult at the present time. The understanding of the role of persistent viral infections may add to understanding of immune defense mechanisms in the animal host.
Smith, Malcolm H. AI 52518 Montana State University $49,000	To define and study factors important in the development of RSV bronchiolitis or pneumonia in an infant animal model, the calf. To study the role of immunologic factors in resistance to RSV infection and disease. To evaluate vaccines and/or antiviral substances in the model. Definition and standardization of the calf model is being completed.
Wright, Peter F. AI 22507 Vanderbilt University $172,000 (75%)	To conduct clinical evaluations of respiratory virus vaccines in infants and children. Surveillance for all infections in a population of children, 6 weeks to 3 years old, continues. Small studies with an attenuated parainfluenza type 3 virus vaccine showed that it was overly attenuated in seropositive 3 year old children,

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Wright, Peter F. (cont.) AI 22507 Vanderbilt University $172,000 (75%)	but was infective in seronegative younger children. Vaccine needs to be evaluated in additional infants. See also Influenza (25%).

PROGRAM: BACTERIAL DISEASES - PNEUMONIA - PNEUMOCOCCAL

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Austrian, Robert AI 32517 University of Pennsylvania $0 (funded in FY75)	To conduct studies on the epidemiology and immunology of pneumococcal infection and to conduct a field trial to demonstrate efficacy of candidate multivalent pneumococcal polysaccharide vaccines. To perform serologic assays on sera from the trial conducted under subcontract and on sera from the Kaiser Permanente efficacy trial (NIAID contract). An efficacy study at Dorothea Dix Hospital (subcontract) has been completed and the data are being analyzed. Technical assistance is provided by this contractor to the Kaiser field trial (supported by NIAID) and to field trials in South Africa (not supported by NIAID). Efficacy of polysaccharide vaccine in preventing pneumococcal pneumonia has been shown in the South African studies.
Freid, Marvin A. AI 22513 Kaiser Permanente $318,000	To conduct a field trial to determine the efficacy of a dodecavalent pneumococcal polysaccharide vaccine in preventing pneumonia in adults age 45 and older. More than 13,600 volunteers have received either the vaccine or a saline placebo. No serious reactions have been observed and antibody response to all the monovalent components has been good. Data are currently being evaluated to determine whether the vaccine shows efficacy in preventing type-specific pneumococcal pneumonia and also the effect of the vaccine in preventing pneumonia in general.

PROGRAM: BACTERIAL DISEASES - PNEUMONIA
MYCOPLASMA

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Couch, Robert B. AI 32506 Baylor College of Medicine $42,000 (15%)	To evaluate an inactivated Mycoplasma pneumoniae vaccine (OSU-1A) by developing and testing experimental infection challenge systems for M. pneumoniae following immunization. Studies have shown the vaccine to be antigenic and safe in humans and stable during storage at 4°C. Large pools of broth and tissue culture grown Mycoplasma pneumoniae have been prepared and stored. They will be used for experimental infection studies in humans by intranasal and small particle aerosol inoculation. See also Other Viral Respiratory Infections (70%) and Antiviral Substances - Clinical Evaluation (15%)
Senterfit, Laurence B. AI 02065 Cornell University Medical College $10,000	To serve as a serologic testing laboratory for the study of the antibody responses of volunteers in the University of Virginia/Parris Island Mycoplasma pneumoniae vaccine trial (supported by NIAID contract). Aliquots of all sera collected in the trial (30,000 specimens) have been sent to this reference laboratory. Preliminary serologic response along with data on Mycoplasma isolations indicate that greater numbers of recipients of placebo had disease than did recipients of vaccine. Following completion of the serologic tests and analysis of the antibody responses, the data from this study will complement that from the University of Virginia for evaluation efficacy of the vaccine.

PROGRAM: BACTERIAL DISEASES - PNEUMONIA MYCOPLASMA

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Wenzel, Richard P. AI 42512 University of Virginia $44,000	To determine the efficacy of a formalin-inactivated Mycoplasma pneumoniae vaccine (OSU-1A) in preventing Mycoplasma pneumoniae Approximately 8,000 volunteers at the Parris Island Marine Corps Recruit Camp received either the OSU-1A vaccine or a saline placebo in the summer and fall of 1974. Analysis of data available to date shows that the vaccine is acceptable and safe in a military group at high risk of acquiring M. pneumoniae illness. The vaccine has also been shown efficacious with a 52 percent reduction in pneumonia and a 67 percent reduction of M. pneumoniae specific pneumonia. Complete analysis of the data, including results of the serologic testing underway at Cornell University (under NIAID contract) will determine the final efficacy figure.

PROGRAM: BACTERIAL DISEASES - MENINGITIS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Anderson, Porter and Smith, David H. AI 12196 Children's Hospital, Boston $138,000	To develop and test candidate vaccine preparations for prevention of Hemophilus influenzae type b infections in infants. To serve as a serologic reference laboratory in support of other IDB contractors performing clinical studies with H. influenzae polyribose phosphate (PRP) vaccine. Past work by this and other contractors has shown that purified PRP is poorly immunogenic in infants. Using an alternate method of purification, the contractor has prepared a form of PRP (designated PRPc) which is highly immunogenic for weanling rabbits and may be a promising immunogen for use in infants. The material is a complex of PRP, five or more polypeptides, and a trace of lipopolysaccharide. When given to adults, PRPc caused some reactions and good antibody response. Work is continuing to decrease the toxicity of the preparation.
Douglas, R. Gordon, Jr. AI 22503 University of Rochester $46,000 (25%)	To conduct clinical studies to evaluate reactivity and immunogenicity of candidate bacterial vaccine preparations. Hemophilus influenzae type b polyribose phosphate (PRP) vaccine has been evaluated in infants. Children under 18 months of age have been shown to respond very poorly to PRP. This contractor has also studied the serologic response to PRP of patients with invasive H. influenzae type b infection. They have found very little evidence of antibody response in children under 13 months of age; however, almost all patients 24 months of age or older had significant antibody increases. See also Influenza (40%) and Other Viral Respiratory Infections (35%).

PROGRAM: BACTERIAL DISEASES - MENINGITIS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Gold, Ronald AI 22502 University of Connecticut $44,000	To conduct clinical evaluation studies of meningococcal group A and C polysaccharide vaccines in infants. Since the beginning of this contract, 1,534 doses of meningococcal A and/or C vaccine have been given to 452 infants two years of age and under. No significant local or systemic reactions have been observed with the vaccines. Good antibody responses have been elicited in very young infants by meningococcal A vaccine using a two dose regimen. The response of younger infants to group C vaccine is not increased with boostering; in fact, primary immunization at an early age (i.e., 3 months) appears to cause a dampening of the response to vaccine when given later (i.e., 12 months of age). Simultaneous administration of DPT with meningococcal vaccines has no effect on the infant response to A and C polysaccharides.
Gotschlich, Emil C. AI 12502 Rockefeller University $34,000	To serve as a reference testing laboratory to measure (using the radio-immuno assay procedure) the serum antibody response of children and infants to immunization with groups A and C meningococcal polysaccharide vaccines. Assay of serum specimens from IDB meningococcal meningitis contractors is ongoing.
Mäkelä, P. Helena AI 52502 Central Public Health Laboratory $100,000	To conduct an efficacy trial of the meningococcal group A polysaccharide vaccine in children three months to six years of age using the Hemophilus influenzae type b polysaccharide vaccine as a control. Approximately 50,000 children received each of the vaccines in three provinces of Finland in November of 1974. The vaccines were well tolerated. Preliminary data indicate that the meningococcal A vaccine has been effective in preventing group A disease even in the youngest age group vaccinated. The H. influenzae vaccine appears to protect children over 18 months of age against H. influenzae meningitis but is ineffective in younger children.

PROGRAM: BACTERIAL DISEASES - MENINGITIS BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
McIntosh, Kenneth AI 22501 University of Colorado $32,000 (40%)	To conduct clinical evaluation studies of Hemophilus influenzae type b polyribose phosphate (PRP) and meningococcal groups A and C polysaccharide vaccine in children. This contractor has shown that children under age two respond poorly to the H. influenzae vaccine and that a poor antibody response to PRP is seen in response to disease in children of this age group. Young children respond well to meningococcal group A vaccine with response to meningococcal group C vaccine being somewhat poorer. See also Influenza (30%) and Other Viral Respiratory Infections (30%).
Wehrle, Paul F. AI 22506 University of Southern California $12,000 (for three months)	To conduct clinical evaluation studies of the Hemophilus influenzae type b polysaccharide and meningococcal A and C polysaccharide vaccines in infants. To set up a disease surveillance system and study the incidence of bacterial meningitis in Los Angeles County as a foundation for a possible future vaccine trial. Studies on clinical evaluation of polysaccharide vaccines are being completed. Final data are being analyzed and are expected to support preliminary observations that infant response to the H. influenzae vaccine is very poor and that low but measurable responses are seen to meningococcal groups A and C vaccines. With regard to the surveillance study: as of the end of 1975, 146 out of 194 hospitals in Los Angeles County were enrolled in the study. One hundred and eighty cases of bacterial meningitis have been seen in these participating hospitals. Data on causal organism, age group, census tracts, etc., are being analyzed at the present time.

PROGRAM: BACTERIAL DISEASES - OTITIS MEDIA BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Howie, Virgil M. AI 52537 Huntsville Hospital (Alabama) $0 (funded in FY75)	To conduct a trial of the efficacy of an octavalent pneumococcal polysaccharide vaccine in preventing pneumococcal otitis media in infants and young children who have had previous middle ear infections. Approximately 200 children are to receive either the octavalent vaccine serotypes commonly causing otitis media or a control vaccine containing seven polysaccharides of other pneumococcal types less important in middle ear disease. At the present time, 76 children have been enrolled in the study and have been vaccinated. No data on antibody response of efficacy of vaccine are available at this point.
Klein, Jerome O. AI 52536 Boston City Hospital $132,000	To conduct a trial of the efficacy of an octavalent pneumococcal polysaccharide vaccine in preventing pneumococcal otitis media in infants and young children who have had previous middle ear infections. Approximately 200 children will receive either the octavalent vaccine containing polysaccharides of serotypes commonly causing otitis media or a control vaccine containing seven polysaccharides of other pneumococcal types less important in middle ear disease. Under this protocol, children are enrolled in the project at birth and vaccinated after three or more bouts of otitis media and after reaching at least six months of age. To date, 752 infants have been enrolled and four have received the pneumococcal vaccine without untoward reactions. No data on serologic response or efficacy are available at this time.
Quie, Paul G. AI 52533 University of Minnesota $88,000	To develop an animal model in which to study the pathogenesis and immunology of pneumococcal otitis media and the efficacy of pneumococcal polysaccharide vaccines in preventing experimentally induced middle ear disease. To study the relative importance of serum and middle ear fluid antibody in pneumococcal otitis media. The contractor has achieved pneumococcal infections in the middle ear of their chinchilla model and have found the immunopathologic responses of the animals to different serotypes

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Quie, Paul G. (cont.) AI 52533 University of Minnesota $88,000	to be very different. Antibody responses also differed. Further studies of other pneumococcal serotypes, including protection experiments using polysaccharide vaccines, are in progress.
Schiffman, Gerald AI 42541 State University of New York (Downstate Medical Center) $83,000	To serve as a serologic testing laboratory in support of the total pneumococcal vaccine program. In particular, to support studies on safety and antigenicity of pneumococcal polysaccharides in infants and efficacy of an octavalent pneumococcal vaccine for prevention of otitis media in infants. The contractor measures antibodies in serum samples to a large number of pneumococcal polysaccharides using the radio-immuno assay procedure. Radioactivity labeled antigens are supplied to other Branch contractors who perform their own assays. Serologic testing is a continually ongoing process. A method for subtyping pneumococci by the radio-immuno assay has been developed by this contractor.
Sell, Sarah H. AI 52538 Vanderbilt University $0 (funded in FY75)	To conduct studies to determine the optimum dose and booster regimen for maximum antibody response of infants and young children to the octavalent pneumococcal polysaccharide vaccine. Data from a previous contract indicate that infants under one year of age respond inconsistently to these vaccines. This contract will extend that study to a larger number of children and will examine more varied dose and booster regimens. At the present time, 51 children have been enrolled in the study and 29 have received one of the various dose levels of vaccine with no untoward reactions. No data on antibody response are available at this time.

PROGRAM: BACTERIAL DISEASES - STREPTOCOCCAL INFECTIONS

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Fox, Eugene N. AI 32519 University of Chicago $11,000	To determine the effectiveness of streptococcal M-protein vaccines in prevention of infection in human challenge experiments. To prepare rabbit antisera to streptococcal M protein preparations produced by several collaborative investigators in order to determine the best method of antigen production (this is being done as a subcontract of this project). In studies of types 1, 3, and 12, data indicate that protection can be obtained against experimental challenge by immunizing with type specific M-protein via the parenteral and intranasal routes. Concerns about the use of human challenge experiments, the lack of purity of the current preparations and the complexity of serologic types which would be required in a candidate vaccine have led to a planned phase out of this contract.

PROGRAM: ANTIVIRAL SUBSTANCES - NONCLINICAL RESEARCH AND DEVELOPMENT

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Easterday, Bernard C. AI 02132 University of Wisconsin $68,000	To provide a viral disease animal model which closely mimics a disease state in man so as to allow evaluation of promising antivirals as soon as they are identified. This contract is a component of the Cooperative Antiviral Testing Group which tested several antivirals and continues baseline studies with interferon and its inducers. This particular contract includes swine influenza, transmissible gastroenteritis and pseudorabies viruses in the piglet.
Friedman-Kien, Alvin E. AI 02131 New York University Medical Center $104,000	To provide a viral disease animal model which closely mimics a disease state in man so as to allow evaluation of promising antivirals as soon as they are identified. This contract is a component of the Cooperative Antiviral Testing Group which tested several antivirals and continues baseline studies with interferon and its inducers. This particular contract includes vaccinia, fibroma and herpesviruses as dermatological infections in the mouse and rabbit.
Gerone, Peter J. AI 52525 Tulane University $94,000	To provide a viral disease animal model which closely mimics a disease state in man so as to allow evaluation of promising antivirals as soon as they are identified. This contract is a component of the Cooperative Antiviral Testing Group which tested several antivirals and continues baseline studies with interferon and its inducers. This particular contract includes two models: influenza A virus and the squirrel monkey and Delta herpesvirus and the patas monkey.
Glasgow, Lowell A. AI 42524 University of Utah $93,000	To provide a viral disease animal model which closely mimics a disease state in man so as to allow evaluation of promising antivirals as soon as they are identified. This contract is a component of the Cooperative Antiviral Testing Group which tested several antivirals and continues baseline studies with interferon and its

PROGRAM: ANTIVIRAL SUBSTANCES - NONCLINICAL RESEARCH AND DEVELOPMENT

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Glasgow, Lowell A. (cont.) AI 42524 University of Utah $93,000	inducers. This particular contract includes herpesvirus, cytomegalovirus and EMC virus in the mouse.
Greenberg, Stephen B. AI 42530 Baylor College of Medicine $50,000	To continue studies to determine why large amounts of interferon when administered intranasally are needed for effective inhibition of rhinovirus whereas doses four orders of magnitude smaller are totally inhibitory in human tissue culture, and to design ways to decrease by several orders of magnitude the dosage required for effective viral inhibitions.
Grossberg, Sidney E. AI 32530 Medical College of Wisconsin $0	The contractor has reviewed a number of promising biological inducers of interferon and produced them in quantity for animal studies and for biochemical analysis. Although the results are impressive, none of the inducers were found to have clinical potential. Contract terminating.
Grossberg, Sidney E. AI 42514 Medical College of Wisconsin $39,000	To lyophilize diploid human fibroblast cell interferon and prepare in ampule form as a research reference reagent; this includes stability studies and periodic verifications of titer.
Grossberg, Sidney E. AI 42520 Medical College of Wisconsin $63,000	To study partially purified human interferon in an attempt to stabilize it. Such studies are of importance since interferon loses its titer as it is purified. If this material is to be used clinically, methodologies for its stabilization should be found. This study has yielded good data and is currently in its final phase.
McCoy, Thomas A. AI 52521 Lee-Mac $42,000	Using optimal induction procedures, this contractor is designing and testing hardware for large-scale production of human interferon. These techniques will then become available to producers of clinical material.

PROGRAM: ANTIVIRAL SUBSTANCES - NONCLINICAL RESEARCH AND DEVELOPMENT

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Baucke, Kurt AI 52529 Medical College of Pennsylvania $43,000	To develop monospecific antibodies against human leucocyte and fibroblast interferons. This is being accomplished by induction of cells to produce antisera to interferon, mutagenizing cells and selecting those which are producing only one antibody, that directed against interferon. This material will be useful in interferon purification (using affinity chromatography columns) and mechanism studies.
Scott, Fredric W. AI 52527 Cornell University $96,000	To provide a viral disease animal model which closely mimics a disease state in man so as to allow evaluation of promising antivirals as soon as they are identified. This contract is a component of the Cooperative Antiviral Testing Group which tested several antivirals and continues baseline studies with interferon and its inducers. This particular contract includes as an animal model the cat with feline picornavirus (calicivirus) and feline herpesvirus; both are respiratory viruses.
Vilcek, Jan T. AI 02169 New York University Medical Center $97,000	To develop a more economically feasible method for the production of human interferon which would be acceptable for use in humans. Techniques have been developed wherein production from diploid human fibroblast cells equals that of leucocytes. A high yielding diploid cell has been identified and attempts to find others will continue. This contract also produced human interferon for purification studies.
Youngner, Julius S. AI 32529 University of Pittsburgh $36,000 (for six months)	Has studied a variety of biological inducers of interferon to determine clinical potential. Although excellent inducers have been identified, none clearly demonstrate clinical potential. However, one such compound has been found to have long-lasting antiviral effect following one administration. This phenomenon is also being investigated during this phase-out period.

PROGRAM: ANTIVIRAL SUBSTANCES - CLINICAL EVALUATION

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Blank, Harvey AI 52531 University of Miami $103,000 ($33,000 of this amount contributed by Dental Institute)	Component of a multicenter study of the natural history of herpes labialis in humans and double-blind, placebo controlled clinical efficacy trials of disodium phosphonoacetate (PAA) and adenine arabinoside monophosphate (ara-AMP) in herpes labialis. When and if such studies show efficacy, these studies could be extended to genital herpes.
Couch, Robert B. AI 32506 Baylor College of Medicine $42,000 (15%)	To evaluate chemoprophylactic and chemotherapeutic agents against human respiratory virus infections. The prophylactic/therapeutic effect of amantadine and rimantadine will be examined. See also Other Viral Respiratory Infections (70%) and Bacterial Diseases - Pneumonia Mycoplasma (15%).
Freedberg, Irwin M. AI 52530 Beth Israel Hospital (Harvard) $110,000 ($34,000 of this amount contributed by Dental Institute)	Component of a multicenter study of the natural history of herpes labialis in humans. This will be followed by a double-blind, placebo controlled clinical efficacy trial of disodium phosphonoacetate (PAA) and adenine arabinoside monophosphate (ara-AMP) in herpes labialis. When and if such studies show efficacy, these studies could be extended to genital herpes.
Merigan, Thomas C. AI 42527 Stanford University $148,000	To clinically evaluate the role of exogenous interferon against serious varicella viral infection in immunologically compromised patients. The initial studies to evaluate the possible toxicity of interferon are completed and the double-blind study is making excellent progress. An open study to evaluate the role of exogenous interferon in eradicating the chronic hepatitis carrier state in patients is being investigated.

PROGRAM: ANTIVIRAL SUBSTANCES - CLINICAL EVALUATION

BRANCH: IDB

Principal Investigator Contract Number Contractor FY76 Obligations	Objectives and Findings
Overall, James C., Jr. AI 52532 University of Utah $127,000 ($33,000 of this amount contributed by Dental Institute)	Component of a multicenter study of the natural history of herpes labialis in humans. This will be followed by a double-blind, placebo controlled clinical efficacy trial of disodium phosphonoacetate (PAA) and adenine arabinoside monophosphate (ara-AMP) in herpes labialis. When and if such studies show efficacy, these studies could be extended to genital herpes. This contractor will also serve as the central unit for collating the data from the three centers.
Whitley, Richard J. AI 22532 University of Alabama $259,000	To clinically evaluate 9-β-D-arabinofuranosyl adenine against herpes encephalitis, disseminated herpes zoster and serious herpes hominis infections, particularly in immunologically compromised patients. This is an extensive collaborative program involving approximately 20 clinics. The double-blind study is progressing well. Some efficacy was observed in the zoster crossover study, but a straight double-blind study, currently underway, is needed to firmly establish efficacy.

Anderson, P., Pitt, J., and Smith, D. H.: Synthesis and Release of Polyribophosphate by Haemophilus influenzae Type b In Vitro. Infect. Immun. 13: 581-589, 1976.

Arvin, A. M., Yeager, A. S. and Merigan, T. C.: The Effect of Leukocyte Interferon on Urinary Excretion of Cytomegalovirus in Infants. J. Infect. Dis. (In Press) 1976.

Austrian, R.: Pneumococcal Vaccines. In Bowen, W. H., Genco, R. J., and O'Brien, Thomas C. (Eds.) Immunologic Aspects of Dental Caries. Proceedings of a Workshop, Bethesda, Maryland, January 8-9, 1976. Spec. Suppl. to Immunol. Abs., Washington, D. C. Information Retrieval, Inc., pp. 125-130, 1976.

Austrian, R.: Vaccines of Pneumococcal Capsular Polysaccharides and the Prevention of Pneumococcal Pneumonia. In Beers, R. F. and Bassett, E. G. (Eds.): The Role of Immunological Factors in Infectious Allergic and Autoimmune Processes. New York, Raven Press, pp. 79-89, 1976.

Beutner, K., Rizzone, C., DeMello, S., and Ogra, P. L.: Clinical Evaluation of Neuraminidase Monospecific (HEqN2) Recombinant Influenza Vaccine in Children. Develop. Biol. Standard. (In Press) 1976.

Buchanan, T. M., Pearce, W. A.: Pili as a Mediator of the Attachment of Gonococci to Human Erythrocytes. Infect. Immun. (In Press) 1976.

Buchanan, T. M., Pearce, W. A. Schoolnik, G. K., Arko, R. J.: Gonococcal Antigens and Immunity. J. Infect. Dis. (In Press) 1976.

Ch'ien, L. T., Whitley, R. J., Alford, C. A., Galasso, G. J. (Eds.): NIAID Collaborative Antiviral Study: Preliminary Results with ara-A Therapy of Herpes Zoster in the Immunosuppressed. J. Infect. Dis. Suppl. (In Press) 1976.

Chisari, F. V., and Edgington, T. S.: Human T Lymphocyte "E" Rosette Function. I.A. Process Modulated by Intracellular Cyclic AMP. J. Exp. Med. 140: 1122-1126, 1974.

Chisari, F. V., and Edgington, T. S.: Lymphocyte E Rosette Inhibitory Factor: A Regulatory Serum Lipoprotein. J. Exp. Med. 142: 1092-1107, 1975.

Chisari, F. V., Gerin, J. L., Edgington, T. S.: Immunochemistry of the Hepatitis B Virus: I HB Ag Ligand. J. Immunol. 113: 543-553, 1974.

Chisari, F. V., Routenberg, J. A., Edgington, T. S.: Mechanisms Responsible for Defective Human T-Lymphocyte Sheep Erythrocyte Rosette Function Associated with Hepatitis B Virus Infections. J. Clin. Invest. 57: 1227-1238, 1976.

Couch, R. B.: Assessment of Immunity to Influenza Using Artificial Challenge of Normal Volunteers with Influenza Virus. Develop. Biol. Standard. 28: 295-306, 1975.

Douglas, R. G., Jr.: Influenza in Man. In Kilbourne, E. D. (Ed.): The Influenza Viruses and Influenza. New York, Academic Press. 13: 395-447, 1975.

Douglas, R. G., Jr., Bentley, D. W., Betts, R. F., Zaky, D. A., Roth, F. K., Murphy, B. R.: Evaluation of a Temperature Sensitive Influenza Virus in Elderly and Chronically Ill Subjects. Am. Rev. Resp. Dis. 113: 293-300, 1976.

Easterday, B. C.: Animal Influenza. In Kilbourne, E. D. (Ed.): The Influenza Viruses and Influenza. New York, Academic Press. 14: 449-481.

Feingold, D. S., Keleti, G., and Youngner, J. S.: Antiviral Activity of Brucella abortus Preparations: Separation of Active Components. Infect. Immun. 13: 763-767, 1976.

Fitzgerald, T. J., Miller, J. N., Sykes, J. A., and Johnson, R. C.: Tissue Culture and Treponema pallidum. In Johnson, R. C. (Ed.): The Biology of Parasitic Spirochetes. New York, Academic Press, pp. 57-64, 1976.

Fox, E. N.: Group A Streptococcal Vaccines. In Bowen, W. H., Genco, R. J., and O'Brien, T. C. (Eds.): Immunologic Aspects of Dental Caries. Proceedings of a Workshop, Bethesda, Maryland, January 8-9, 1976. Spec. Suppl. Immunol. Abs., Washington, D. C., Information Retrieval, Inc. pp. 137-141, 1976.

Foy, H. M., Wentworth, B., Kenny, G. E., Kloeck, J. M. and Grayston, J. T.: Pneumococcal Isolations from Patients with Pneumonia and Control Subjects in a Prepaid Medical Care Group. Am. Rev. Resp. Dis. 111: 595-603, 1975.

Friedman-Kien, A. E., Fondak, A. A. and Klein, R. J.: Phosphonoacetic Acid Treatment of Shope Fibroma and Vaccinia Virus Skin Infections in Rabbits. J. Invest. Derm. 66: 99-102, 1976.

Galin, M. A., Chowchuvech, E., and Kronenberg, B.: Therapeutic Use of Inducers of Interferon on Herpes Simplex Keratitis in Humans. Ann. Ophthalmol. 8: 72-76, 1976.

Glasgow, L. A.: Advantages and Limitations of Animal Models in the Evaluation of Antiviral Substances. J. Infect. Dis. (In Press) 1976.

Gold, R. and Lepow, M.: Present Status of Polysaccharide Vaccines in the Prevention of Meningococcal Disease. Adv. Pediatr. (In Press) 1976.

Gold, R., Lepow, M. L., Goldschneider, I., Draper, T. L., Gotschlich, E. C.: Clinical Evaluation of Group A and Group C Meningococcal Polysaccharide Vaccines in Infants. J. Clin. Invest. 56: 1536-1547, 1975.

Gotschlich, E. C.: Development of Polysaccharide Vaccines for the Prevention of Meningococcal Diseases. Monogr. Allergy 9: 245, Basel, Karger, 1975.

Gotschlich, E. C.: Unresolved Problems in the Prevention of Meningococcal Meningitis. In Beers, R. F. and Bassett, E. G. (Eds.): The Role of Immunological Factors in Infectious, Allergic, and Autoimmune Processes. New York, Raven Press, p. 91, 1976.

Graves, S. R., Sandok, P. L., Jenkin, H. M., and Johnson, R. C.: Retention of Motility and Virulence of Treponema pallidum (Nichols Strain) In Vitro. Infect. Immun. 12: 1116-1120, 1975.

Greenberg, S. G., Criswell, B. S., Couch, R. B.: Lymphocyte-Mediated Cytotoxicity Against Influenza Virus-Infected Cells: An In Vitro Method. J. Immunol. 115: 601-603, 1975.

Greiff, D., Melton, H., and Row, T. W. G.: On the Sealing of Gas-Filled Glass Ampoules. Cryobiology 12: 1-14, 1975.

Grossberg, S. E., Smith, A. J. and Sedmak, J. J.: Control of the Interferon Mechanism in Hamster-Mouse Heterokaryons. In Geraldes, A. (Ed.): Effects of Interferon on Cells, Viruses and the Immune System. New York, Academic Press, I: 33-53, 1975.

Gwaltney, J. M., Jr., Sande, M. A., Austrian, R. and Hendley, J. O.: Spread of Streptococcus pneumoniae in Families. II. Relation of Transfer of S. pneumoniae to Incidence of Colds and Serum Antibody. J. Infect. Dis. 132: 62-68, 1975.

Hall, C. B., and Douglas, R. G., Jr.: Clinically Useful Method for the Isolation of Respiratory Syncytial Virus. J. Infect. Dis. 131: 1-5, 1975.

Hall, C. B. and Douglas, R. G., Jr.: Practical Community Surveillance of RSV and Influenza. Am. J. Dis. Child. (In Press), 1976

Hall, C. B., Douglas, R. G., Jr., Geiman, J. M.: Quantitative Shedding Patterns of Respiratory Syncytial Virus in Infants. J. Infect. Dis. 132: 151-156, 1975.

Hall, C. B., Douglas, R. G., Jr., Geiman, J. M., and Messner, J. K.: Nosocomial Respiratory Syncytial Virus Infections. N. Engl. J. Med. 293: 1343-1346, 1975.

Hall, C. B., Geiman, J. M., Biggar, R., Kotok, D., I. Hogan, P. M., Douglas, R. G., Jr.: Respiratory Syncytial Virus Infections Within Families. N. Engl. J. Med. 294: 414-419, 1976.

Hall, W. J., Douglas, R. G., Jr., Hyde, R. W., Roth, F. K., Cross, A. S., and Speers, D. M.: Pulmonary Mechanics After Uncomplicated Influenza A Infection. Am. Rev. Resp. Dis. 113: 141-147, 1976.

Hall, W. J., Douglas, R. G., Jr., Zaky, D. A., Hyde, R. W., Richman, D., Murphy, B. R.: Attenuated Influenza Virus in Normal Adults: Role of Pulmonary Function Studies in Vaccine Trials. J. Infect. Dis. 133: 145-152, 1976.

Harmon, M. W., Greenberg, S. G., and Couch, R. B.: Effect of Human Nasal Secretions on the Antiviral Activity of Human Fibroblast and Leukocyte Interferon. Proc. Soc. Exp. Biol. Med. (In Press) 1976.

Harmon, M. W., and Janis, B.: Effects of Cytosine Arabinoside, Adenine Arabinoside, and 6-Azauridine on Rabies Virus In Vitro and In Vivo, J. Infect. Dis. 133: 7-13, 1976.

Harmon, M. W. and Janis, B.: Therapy of Murine Rabies After Exposure: Efficacy of Polyriboinosinic-Polyribocytidylic Acid Alone and in Combination with Three Rabies Vaccines. J. Infect. Dis. 132: 241-249, 1975.

Havell, E. A., Berman, B., Ogburn, C. A., Berg, K., Paucker, K. and Vilcek, J.: Two Antigenically Distinct Species of Human Interferon. Proc. Nat. Acad. Sci. USA 72: 2185-2187, 1975.

Hendley, J. O., Sande, M. A., Stewart, P. M., and Gwaltney, J. M., Jr.: Spread of Streptococcus pneumoniae in Families. I. Carriage Rates and Distribution of Types. J. Infect. Dis. 132: 55-61, 1975.

Jariwalla, R. J., Grossberg, S. E., and Sedmak, J. J.: The Influence of Physico-chemical Factors on the Thermal Inactivation of Murine Interferon. Arch. Virology 49: 261, 1975.

Johnson, P. E., Greenberg, S. B., Harmon, M. W., Alford, C. A., Jr. and Couch, R. B.: Recovery of Applied Human Leukocyte Interferon from the Nasal Mucosa of Chimpanzees and Humans. J. Clin. Microbiol. (In Press) 1976.

Kanra, G. Y. and Vesikari, T.: Cytotoxic Activity Against Rubella-Infected Cells in the Supernatants of Human Lymphocyte Cultures Stimulated by Rubella Virus. Clin. Exp. Immunol. 19: 17-32, 1975.

Kapikian, A. Z., Kim, H. W., Wyatt, R. G., Cline, W. L., Arrobio, J. O., Brandt, C. D., Rodriguez, W. J., Sack, D. A., Chanock, R. M. and Parrott, R. H.: Human Reovirus-like (HRVL) Agent as the Major Pathogen Associated with "Winter" Gastroenteritis - Etiologic, Epidemiologic Studies in Infants, Young Children and Their Contacts. N. Engl. J. Med. (In Press) 1976.

Kasel, J. A., Couch, R. B., Six, H. R., Knight, V.: Antigenicity of Licensed Whole Virion and Subvirion Influenza Vaccines in "High Risk" Persons. Proc. Soc. Exp. Biol. Med. (In Press) 1976.

Kelsey, D. K., Kern, E. R., Overall, J. C., Jr., and Glasgow, L. A.: The Effect of Cytosine Arabinoside and 5-iodo-2'deoxyuridine on a Cytomegalovirus Infection in Newborn Mice. Antimicrob. Agents Chemother. 7: 587-595, 1975.

Kenny, G. E. and Foy, H. M.: Detection and Quantification of Circulating Polysaccharide in Pneumococcal Pneumonia by Immunoelectroosmorphoresis (Counterelectrophoresis) and Rocket Electrophoresis. In Schlessinger, D. (Ed.) Microbiology, 1975. Washington, D. C., American Society for Microbiology. 1: 97-102.

Kern, E. R., Glasgow, L. A., and Overall, J. C., Jr.: Antiviral Activity of an Extract of Brucella abortus: Induction of Interferon and Immunopotentiation of Host Resistance. Proc. Soc. Exp. Biol. Med. (In Press) 1976.

Kern, E. R., Overall, J. C., Jr., and Glasgow, L. A.: Herpesvirus hominis Infection in Newborn Mice: Comparison of the Therapeutic Efficacy of 1-β-D Arabinofuranosylcytosine and 9-β-D Arabinofuranisyladenine. Antimicrob. Agents Chemother. 7: 587-595, 1975.

Kern, E. R., Overall, J. C., Jr., and Glasgow, L. A.: Herpesvirus hominis Infection in Newborn Mice: Treatment with Interferon Inducer Polyinosinic-Polycytidylic Acid. Antimicrob. Agents Chemother. 7: 793-800, 1975.

Kim, H. W., Leikin, S. L., Arrobio, J., Brandt, C. D., Chanock, R. M., and Parrott, R. H.: Cell-Mediated Immunity to Respiratory Syncytial Virus Induced by Inactivated Vaccine or by Infection. Pediatr. Res. 10: 75-78, 1976.

Kim, H. W., Arrobio, J. O., Brandt, C. D., Parrott, R. H., Murphy, B. R., Richman, D. D., and Chanock, R. M.: Temperature Sensitive Mutants of Influenza A Virus. X. Response of Children to the Influenza A/Hong Kong/68-ts-1[E] (H3N2) and Influenza A/Udorn/72-ts-1[E] (H3N2) Candidate Vaccine Viruses and Significance of Immunity to Neuraminidase Antigen. Pediatr. Res. 10: 238-242, 1976.

Kim, H. W., Brandt, C. D., Arrobio, J. O., Chanock, R. M., Parrott, R. H.: Virus Infections in Respiratory Disease Patients During 1974. Clin. Proc. 32: 56-58, 1976.

Klein, R. J.: Isolation of Herpes Simplex Virus Clones and Drug Resistant Mutants in Microcultures. Arch. Virology 49: 73-80, 1975.

Laver, W. G. and Webster, R. G.: Preparation and Immunogenicity of an Influenza Virus Hemagglutinin and Neuraminidase Subunit Vaccine. Virology 69: 511-522, 1976.

Laver, W. G., Downie, J. C. and Webster, R. G.: Diversity of the Antibody Response to the Different Antigenic Determinants on the Hemagglutinin Subunits of Influenza Viruses. J. Immunol. 116: 336-341, 1976.

McMorrow, L. E., Vesikari, T., Wolman, S. R., Giles, J. P. and Cooper, L. Z.: Suppression of the Response of Lymphocytes to Phytohemagglutinin in Rubella. J. Infect. Dis. 130: 464-469, 1974.

Miller, J. N.: Potential for Vaccines for Venereal Diseases. Bull. N.Y. Acad. Med. (In Press) 1976.

Monto, A. S., Bryan, E. R. and Rhodes, L. M.: The Tecumseh Study of Respiratory Illness. VII. Further Observations on the Occurrence of Respiratory Syncytial Virus and Mycoplasma Pneumoniae Infections. Am. J. Epidemiol. 100: 458-468, 1975.

Monto, A. E. and Kioumehr, F.: The Tecumseh Study of Respiratory Illness. IX: Occurrence of Influenza in the Community, 1966-1971. Am. J. Epidemiol. 102: 553-563, 1975.

Mostow, S. R., Flatauer, S. and Martinko, J.: An In Vitro Marker of Attenuation for Live Influenza Virus Vaccine Candidates. International Symposium on Immunity to Infections of the Respiratory System in Man and Animals, London, 1974. Develop. Biol. Standard. 28: 354-362, Basel, Karger, 1975.

Moxon, E. R., Anderson, P., Smith, D. H., Adrianzen, B. T., Graham, G. G., and Baker, R. S.: Antibody Responses to a Combination Vaccine Against Haemophilus influenzae type b, Diphtheria, Pertussis and Tetanus. Bull. WHO 52: 87-90, 1975.

Mozes, L. W. and Vilcek, J.: Distinguishing Characteristics of Interferon Induction with Poly (I)·Poly (C) and Newcastle Disease Virus in Human Cells. Virology 65: 100-111, 1975.

Nath, N., Mazzur, S., Ledman, R. and Fang, C. T.: Purification of Hepatitis B Surface Antigen Using Polyethylene Glycol (PEG), Pepsin and Tween 80. Vox Sang. (In Press) 1976.

O'Reilly, R. J., Anderson, P., Ingram, D. L., Peter, G., Smith, D. H.: Circulating Polyribophosphate in Hemophilus influenzae, Type b Meningitis: Correlation with Clinical Course and Antibody Response. J. Clin. Invest. 56: 1012-1022, 1975.

Overall, J. C., Jr., Kern, E. R., and Glasgow, L. A.: Effective Antiviral Chemotherapy in a Cytomegalovirus Infection of Mice. J. Infect. Dis. (In Press) 1976.

Pabico, R. C., Douglas, R. G., Betts, R. F., McKenna, B. A. and Freeman, R. B.: The Antibody Response to Influenza Vaccination in Renal Transplant Patients: Correlation with Allograph Function. Ann. Intern. Med. (In Press) 1976.

Parrott, R. H., Kim, H. W., Brandt, C. D. and Chanock, R. M.: Potential of Attenuated Respiratory Syncytial Virus Vaccine for Infants and Children. Develop. Standard. 28: 389-399, Basel, Karger, 1975.

Paucker, K., Ogburn, C. A. and Berg, K.: Purification of Interferons by Immune Affinity Chromatography. In Hasegawa, T. (Ed.): Proceedings of the First Intersectional Congress of IAMS. Science Council of Japan, 4: 76-85, 1975.

Paucker, K., Dalton, B. J., Ogburn, C. A., and Torma, E.: Multiple Active Sites on Human Interferons (Interferon Antigens/Heterospecific Protection/Rabbit Cells/Antibodies Against Interferon/Affinity Chromatography). Proc. Nat. Acad. Sci. USA 72: 4587-4591, 1975.

Paucker, K., Berg, K., Ogburn, A. C.: Purification of Mouse Interferon by Antibody Affinity Chromatography. In Geraldes, A. (Ed.): Effects of Interferon on Cells, Viruses and the Immune System. London, Academic Press, VII: 639-655, 1975.

Pestka, S., McInnes, J., Weiss, D., Havell, E. A., and Vilcek, J.: De novo Cell-Free Synthesis of Human Interferon. Ann. N.Y. Acad. Sci. (In Press) 1976.

Pestka, S., McInnes, J., Havell, E. A., and Vilcek, J.: Cell-Free Synthesis of Human Interferon. Proc. Nat. Acad. Sci. USA 72: 3898-3901, 1975.

Pons, M. W.: Influenza Virus RNA(s). In Kilbourne, E. D. (Ed.): The Influenza Viruses and Influenza. New York, Academic Press, 6: 145-170, 1975.

Robbins, R., and Galin, M. A.: A Model for Steroid Effects in Herpes Keratitis. Arch. Ophthalmol. 93: 828-830, 1975.

Sandok, P. L., Jenkin, H. M., Graves, S. R., and Knight, S. T.: Retention of Motility of Treponema pallidum (Nichols Virulent Strain) in an Anaerobic Cell Culture System and in a Cell-Free System. J. Clin. Microbiol. 3: 72-74, 1976.

Scott, J. V., Dreesman, G. R., Spira, G., and Kasel, J. A.: Radioimmunoassay of Human Serum Antibody Specific for Adenovirus Type 5-Purified Fiber. J. Immunol. 115: 124-127, 1975.

Seeff, L. B., Wright, E. C., Zimmerman, H. J., McCollum, R. W., Members of VA Hepatitis Cooperative Studies Group: VA Cooperative Study of Post-Transfusion Hepatitis, 1969-1974: Incidence and Characteristics of Hepatitis and Responsible Risk Factors. Am. J. Med. Sci. 270: 355-362, 1975.

Seeff, L. B., Zimmerman, H. J., Wright, E. C., Felsher, B. F., Finkelstein, J. D., García-Pont, P., Greenlee, H. B., Dietz, A. A., Hamilton, J., Koff, R. S., Leevy, C. M., Kiernan, T., Tamburro, C. H., Schiff, E. R., Vlahcevic, Z., Zemel, R., Zimmon, D. S., Nath, N.: Efficacy of Hepatitis B Immune Serum Globulin After Accidental Exposure. Lancet: 939-936, 1975.

Sehgal, P. B., Tamm, I., and Vilcek, J.: Human Interferon Production: Superinduction by 5, 6-Dichloro-1-β-ribofurano-sylbenzimidazole. Science 190: 282-284, 1975.

Sehgal, P. B., Tamm, I., and Vilcek, J.: On the Mechanism of Enhancement of Human Interferon Production by Actinomycin D and Cycloheximide. Virology 70: 256-259, 1976.

Sehgal, P. B., Tamm, I., and Vilcek, J.: Regulation of Human Interferon Production. I. Superinduction by 5,6-Dichloro-1-β-ribofurano-sylbenzimidazole. Virology 70: 532-541, 1976.

Simpson, R. W., Iinuma, M.: Recovery of Infectious Proviral DNA from Mammalian Cells Infected with Respiratory Syncytial Virus (ribovirus/temperature-sensitive mutant/nonvirogenic cells/DNA transfection). Proc. Nat. Acad. Sci. USA 72: 3230-3234, 1975.

Simpson, R. W., Iinuma, M., and Guskey, L. E.: Establishment and Detection of Animal DNA Proviruses in Cell Culture Systems. ICN-UCLA Symposium on Animal Virology, Squaw Valley, Cal., March 15-19, 1976. Virology (In Press) 1976.

Sloyer, J., Cote, C., Howie, V., Ploussard, J., Schiffman, G. and Johnson, R.: Immune Response to Acute Otitis Media in Children. III. Relation to Clearing of Middle Ear Fluid. J. Clin. Microbiol. (In Press) 1976.

Steffenhagen, K. A., Easterday, B. C., and Galasso, G. J.: Evaluation of 6-Azauridine and 5-Iododeoxyuridine Activity in Experimental Viral Infections. J. Infect. Dis. (In Press) 1976.

Tiku, M. L., Beutner, K. R., Carmody, P., Sultz, H. A., Kaul, B. L., and Ogra, P. L.: Hepatitis B Infection in Health Care Personnel of an Institution for Mentally Handicapped Children and Adults. J. Clin. Microbiol. (In Press) 1976.

Tiku, M. L., Beutner, K. R., Ramirez, R. I., Dienstag, J. L., Sultz, H. A., and Ogra, P. L.: Distribution and Characteristics of Hepatitis B Antigen (HB_SAg) in Body Fluids in Institutionalized Children and Adults. J. Infect. Dis. (In Press) 1976.

Todd, J. K., and Bruhn, F. W.: Severe Haemophilus influenzae Infections. Am. J. Dis. Child. 129: 607-611, 1975.

Vesikari, T., Kanra, Guler Y., Buimovici-Klein, E. and Cooper, L. Z.: Cell-Mediated Immunity in Rubella Assayed by Cytotoxicity of Supernatants from Rubella Virus-Stimulated Human Lymphocyte Cultures. Clin. Exp. Immunol. 19: 33-43, 1975.

Vilcek, J., and Havell, E. A.: Interferon Production as a Model of Induced Protein Synthesis in Eukaryotic Cells. In Geraldes, A. (Ed.): Effects of Interferon on Cells, Viruses and the Immune System. London, Academic Press, II: 171-190, 1975.

Vilcek, J., Havell, E. A., and Kohase, M.: Superinduction of Interferon with Metabolic Inhibitors: Possible Mechanisms and Practical Application. J. Infect. Dis. (In Press) 1976.

Vilcek, J., Havell, E. A., Mozes, L. W., Berman, B.: Interferon Induction with Viruses and Polynucleotides. In Hasegawa, T. (Ed.): Proceedings of the First Intersectional Congress of IAMS. Science Council of Japan, 4: 65-75, 1975.

Vilcek, J., Havell, E. A., and Yamazaki, S.: Antigenic, Physico-chemical and Biologic Characterization of Human Interferons. Ann. N.Y. Acad. Sci. (In Press) 1976.

Waldman, R. H., Lee, J. D., Polly, S. M., Dorfman, A., and Fox, E. N.: Group A Streptococcal M Protein Vaccine: Protection Following Immunization Via the Respiratory Tract. Develop. Biol. Standard. 28: 429-434, 1975.

Webster, R. B., Kasel, J. A., Couch, R. B., Laver, W. G.: Influenza Virus Subunit Vaccine. II. Immunogenicity and Original Antigenic Sin in Man. J. Infect. Dis. (In Press) 1976.

Whitley, R. J., Ch'ien, L. T., Dolin, R., Galasso, G. J., Alford, C. A. (Eds.): NIAID Collaborative Antiviral Study: Adenine Arabinoside Therapy of Herpes Zoster in the Immunosuppressed. N. Engl. J. Med. 294: 1193-1199, 1976.

Winkelstein, J. A., Bocchini, J. A., Jr., and Schiffman, G.: The Role of the Capsular Polysaccharide in the Activation of the Alternative Pathway by the Pneumococcus. J. Immunol. 116: 367-370, 1976.

Wright, P. F., Sell, S. H., Shinozaki, T., Thompson, J., Karzon, D. T.: Safety and Antigenicity of Influenza A/Hong Kong/68-ts-1[E] (H_3N_2) Vaccine in Young Seronegative Children. J. Pediatr. 87: 1109-1116, 1975.

Wright, P. F., Sell, S. H., Thompson, J. and Karzon, D. T.: Clinical Reactions and Serologic Response Following Inactivated Monavalent Influenza type B Vaccine in Children and Infants. J. Pediatr. 88: 31-35, 1976.

Wright, P. F., Shinozaki, T., Fleet, W., Sell, S. H., Thompson, J., Karzon, D. T.: Evaluation of a Live, Attenuated Respiratory Syncytial Virus Vaccine in Infants. J. Pediatr. (In Press) 1976.

Baron, S., and Galasso, G. J.: Antiviral Agents. In Heinzelman, R. V. (Ed.): Annual Reports in Medicinal Chemistry, New York, Academic Press, 10: 161-171, 1975.

Ch'ien, L. T., Whitley, R. J., Alford, C. A., Galasso, G. J. (Eds.): NIAID Collaborative Antiviral Study: Preliminary Results with ara-A Therapy of Herpes Zoster in the Immunosuppressed. J. Infect. Dis., Suppl. (In Press) 1976.

Galasso, G. J. and Payne, F. J.: Chemotherapy of Viral Diseases: Present Status and Future Prospects. In Kurstak, E. (Ed.): Comparative Diagnosis of Viral Diseases. New York, Academic Press (In Press) 1976.

Steffenhagen, K. A., Easterday, B. C., and Galasso, G. J. Evaluation of 6-Azauridine and 5-Iododeoxyuridine Activity in Experimental Viral Infections. J. Infect. Dis. (In Press) 1976.

Whitley, R. J., Ch'ien, L. T., Dolin, R., Galasso, G. J., Alford, C. A., Jr. (Eds.): NIAID Collaborative Antiviral Study: Adenine Arabinoside Therapy of Herpes Zoster in the Immunosuppressed. N. Engl. J. Med., 294: 1193-1199, 1976.

Choppin, P. W., et al: Genetics, Replication, and Inhibition of Replication of Influenza Virus -- Summary of Influenza Workshop VII. J. Infect. Dis. 132: 713-723, 1975.

Couch, R. B., Jackson, G. G.: Antivirals in Influenza -- Summary of Influenza Workshop VIII. J. Infect. Dis. (In Press) 1976.

Craighead, J. E.: Report of a Workshop: Disease Accentuation after Immunization with Inactivated Microbial Vaccines. J. Infect. Dis. 131: 749-753, 1975.

Pagano, J. S.: Infections with Cytomegalovirus in Bone Marrow Transplantation: Report of a Workshop. J. Infect. Dis. 132: 114-120, 1975.

INFECTIOUS DISEASE BRANCH WORKSHOPS
FOR PROGRAM REVIEW, DEVELOPMENT OR EVALUATION

Date	Meeting	Purpose
April 9, 1975	A workshop was held on streptococcal M Protein as an immunogen	Program Review and Development
August 6, 1975	A workshop was held to discuss Candidate Populations for Hepatitis B Vaccine Trials	Program Development
October 23, 24, 1975	A workshop was held on Current Understanding of Persistent Viral Infections and Their Implications in Human Disease	State of the Art in relation to Program Development
October 25, 1975	The Infectious Disease Committee reviewed implications of persistent viral infections as applied to attenuated vaccines. Also reviewed a proposal on Bacterial Sepsis and Meningitis of the Newborn	Program Review
December 17, 1975	A workshop was held on Hepatitis B Immune Globulin. Jointly with NIAID, NHLI, CDC and BoB.	Review and Evaluation of Current Research
January 15, 16, 1976	The Infectious Disease Committee reviewed IDB's Influenza Program. Also discussed plans for expected pandemic in late 1970s.	Program Review and Development
February 20, 1976	An Influenza Workshop was sponsored jointly by the NIAID, BoB and CDC.	Review Possiblity of Influenza Pandemic. Program Development
March 29-April 1, 1976	A meeting on the Current Status and Prospects for Improved and New Bacterial Vaccines was sponsored jointly by NIAID and the BoB.	State of the Art Review in Relation to Program
April 1, 1976	The Infectious Disease Committee reviewed the Bacterial Vaccines Program.	Program Review and Evaluation

IDB Workshops (cont.)

Date	Meeting	Purpose
April 15, 1976	A meeting, jointly sponsored by the NIAID, BoB, and CDC, was held to discuss Coordinated Efforts for Influenza Research.	Plans for vaccine testing.
June 21, 1976	A meeting was held to discuss the Influenza A/NJ/76 Vaccine Evaluation Studies. Sponsored jointly by NIAID, BoB, DoD, and CDC.	Plans for Vaccine Manufacture and Distribution.

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OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID

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Summary of Program
Laboratory and Clinical Research, NIAID
July 1, 1975 through June 30, 1976

Office of the Scientific Director

The projects that constitute the Laboratory and Clinical Research Program of the National Institute of Allergy and Infectious Diseases are reported and summarized by individual Laboratories, in the pages that follow. The program constitutes intensive and productive research, by both basic and targeted approaches, on problems of immunology and allergy, and of microorganisms and the diseases that they cause.

The Office of the Scientific Director is responsible for the direction and administration of intramural research comprising the above. During most of this year, the responsibility was passed from Dr. John Seal to Dr. Roger Cole (Chief, LSD) as Acting Scientific Director, because of the former's successive acquisition of new duties--first as Acting Director on the retirement in July of Dr. Dorland Davis, and subsequently, in the new post of (Acting) Deputy Director on the arrival in November of the new Director, Dr. Richard Krause. The selection of a new permanent Scientific Director for L&CR is expected before July of 1976. All staff of the Office of the Scientific Director have provided invaluable advice and assistance to the Acting Scientific Director in maintaining the level of operations during a period of change and fiscal uncertainty. The situation was somewhat relieved by the Congressional override in January of the President's veto of the House-Senate Appropriations bill, resulting in an increase to L&CR of $835,000 for other objects, and a restoration of three positions in budgeted ceiling. However, increased costs in contracts and their overheads, and inflation in costs of laboratory supplies and animals, necessitated transfer of additional funds from the Director's Reserve--which may yet not entirely offset projected cost overruns for operations conducted at approximately last year's level of effort.

The Animal Care Section, OSD, in its mission of consolidated animal care for all constituent Laboratories, is functioning well in conjunction with the newly-reconstituted Animal Care Committee. Legal standards for animal care have been met, implementation of training programs for animal caretakers has progressed, and the intramural level of satisfaction with animal care and procedures is greatly improved. The Section Head, Mr. Paul Koger, unexpectedly retired in January after more than 30 years of government service, but has been most ably replaced, on an acting basis thus far, by Mr. Carter Smith.

Scientists remaining assigned to OSD, following laboratory reorganizations discussed in prior years, have continued their service. Dr. Sanford Stone, in continued collaboration with investigators at Albert Einstein College of Medicine, has used inbred guinea pigs to study pathogensis of multiple sclerosis-like (demyelinating) autoimmune encephalitis induced by injection of central nervous system tissues or proteins; and its suppression clinically, though not histologically, by immune deviation techniques. A similar syndrome of autoimmune neuritis, induced by peripheral nerve

tissues, shows a similar age and strain dependence and is under continuing study. Dr. Roy Repaske has extended his investigations of the role of carbon dioxide (bicarbonate) in cell growth. Regulation of growth rate, proportional to CO_2 concentrations of which the optimal differ by type of cell and phase of growth, was previously shown for several bacteria (Hydrogenomonas eutropha, Bacillus megaterium, Streptococcus sanguis) and has now been extended to include Escherichia coli and both normal and transformed eukaryotic cell lines (WI-38 and WI-18, respectively) in tissue culture. The phenomenon, previously largely unrecognized, thus seems to be a general one; and present efforts are directed to identification of the metabolic reaction involved and the intracellular level, site, and mechanisms of control.

The Board of Scientific Counselors met at Bethesda in early December and reviewed the Laboratory of Clinical Investigation. After an initial day's presentations by LCI staff members, visits to the Laboratory and individual discussions with investigators on the second day were followed by a closed meeting of the Board with the Acting Scientific Director, Acting Deputy Director, Director, NIAID, and the assistant Director for Intramural Affairs, NIH. The Laboratory was rated good to excellent overall: the Board cited, particularly, outstanding leadership by the Clinical Director, outstanding and productive personnel, and the high quality of training given and received. A feature of the meetings was the augmented use of ad hoc consultants--of which four, in addition to two members of the NIAID Advisory Council, participated. Success of the procedure, which in effect enhances the aspects of peer review in the meetings, led to Board suggestions for a new meeting format. Board members and the selected consultants will receive written program and project descriptions and projections in advance, will visit the laboratories in advance, and will then meet for purposes of discussion and assessment without a need for individual investigator presentations unless clarifications are needed. This format will be tested at the next meeting, scheduled for a June 1976 review of the Laboratory of Parasitic Diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00131-09-OSD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Hypersensitivity in Histocompatible Inbred Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. H. Stone Head, Immunology Section OSD NIAID

COOPERATING UNITS (if any)

Dr. Cedric S. Raine
Dr. David H. Snyder
Division of Neuropathology
Albert Einstein College of Medicine
New York, N.Y.

LAB/BRANCH

Office of the Scientific Director

SECTION

Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1

SUMMARY OF WORK (200 words or less - underline keywords)

For some years we have been using the unique tool of inbred guinea pigs available at the NIH for investigation of particular areas of the immune response concerned with mechanisms of hypersensitivity and protection against infectious diseases. Advantages lie in the ability to perform viable adoptive transfers of lymphoid cells and in the capacity to study genetic factors in the hypersensitivity phenomena. For these reasons, we have concentrated recently on the pathogenesis of an age-dependent, strain-dependent chronic autoimmune encephalomyelitis which is strongly reminiscent of multiple sclerosis in man and on the prevention and treatment of this condition using various tolerance-inducing techniques, in particular immune deviation approaches.

Project Description:

Immune deviation (ID) techniques which involve the injection of the antigen associated with an inappropriate adjuvant or without adjuvants prior to challenge of the animals with the antigen incorporated in complete Freund's adjuvants (CFA) are able to inhibit the appearance of chronic (CAE) as well as acute (EAE) autoimmune encephalomyelitis in guinea pigs. However, the mechanism of the inhibition may differ from that of classic ID because the duration of the refractive state is considerably shorter. Methods of suppression of EAE involving injection of purified myelin basic protein (MBP) were attempted for development of protocols applicable to CAE. Thus, guinea pigs injected with MBP starting on the seventh day post-challenge with spinal cord incorporated in CFA remained clinically normal for five months following termination of treatment. The clinical results contrasted with the fact that, histologically, central nervous system (CNS) lesions were still present which could not be distinguished from those in controls with typical EAE. In time, suppressed animals showed lesions which were typical of CAE where remyelination was a predominant feature. This separation of clinical from histological findings has also been seen in genetically resistant strain 2 guinea pigs which remain healthy despite the collection of mononuclear cells in CNS foci. The suppression and the genetic deficiency might then both be found at later steps in the pathogenesis of the diseases.

When peripheral (PNS) nerve is used instead of CNS material for attempted induction of experimental autoimmune neuritis (EAN), juvenile strain 13 guinea pigs have shown dramatic morphological changes associated with chronic demyelination in spite of the fact of absence of clinical disease. The major areas of involvement in the PNS were related to the proximal regions of spinal nerve roots. Extensive PNS remyelination and onion bulb formation were seen. This latter finding, as well as the observation of spinal cord invasion by Schwann cells, suggests a recurrent subclinical disease. The differing susceptibilities of adult and juvenile strain 13 animals to PNS (as previously shown to CNS) antigens and their significance to the study of human neuropathies are under continuing investigation.

Publications:

Raine, C.S., Snyder, D.H., Stone, S.H. and Valsamis, M.P.: Chronic Experimental Encephalomyelitis in Inbred Guinea Pigs. In VIIth International Congress of Neuropathology. Budapest, Hungary Akadeniai Kiado, 1975, pp. 153-155.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI-00132- 09 OSD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Growth Regulation in Microorganisms and in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Roy Repaske OSD NIAID

COOPERATING UNITS (if any)

Dr. K. K. Takemoto LVD NIAID

LAB/BRANCH
Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 11/12	1	11/12

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year we have demonstrated that Escherichia coli growth, both in aerobically and in anaerobically cultures, was under CO_2 regulation. The rate of growth was proportional to CO_2 concentration with saturation occurring at 0.02-0.03% CO_2. The influence of CO_2 on growth was also demonstrated with eucaryotic cells represented by normal (WI-38) and transformed (WI-18) embryonic lung cell lines. Each of the mammalian cell lines had dramatically different optimal CO_2 and O_2 concentrations. By culturing WI-38 under the appropriate gas atmosphere, we were able to produce cultures from inocula several orders of magnitude smaller than was formerly possible, and we successfully doubly cloned WI-38 in successive passages. The CO_2 effect on growth of several bacteria reported previously and the examples above constitute a phylogenetic and nutritionally diverse sample of organisms. Each type of cell responded similarly. In every instance, CO_2 (bicarbonate) was required to initiate growth. Once initiated, growth continued to be CO_2 (bicarbonate) dependent. It is particularly significant that the rate of growth was regulated by the concentration of CO_2 (bicarbonate). The uniform response suggests the phenomenon of CO_2 regulation is a general one. The objective of the project is to identify the metabolic reaction controlled by CO_2 (bicarbonate) and determine the mechanism by which this reaction is capable of regulating growth.

Project Description:

The objective of this project is (1) to determine if CO_2 regulation of growth is a general characteristic for all types of cells (procaryots and eucaryots), (2) to identify the metabolic system in which the participation of CO_2 is required for control of growth and (3) to investigate the mechanism by which this system regulates cell growth.

Methods Employed:

The objectives will be accomplished by using two general approaches simultaneously: (1) physiological growth studies to define the responses and to characterize the CO_2 effect on whole cells, and (2) biochemical studies to identify the enzyme or enzyme system involved to determine the mechanism by which the enzyme or its product function in a regulatory capacity. We have devised a system for growing cultures which accurately establishes and maintains a gas atmosphere of three gases in any desired proportions; the proportions of gases can be rapidly changed at any time during the experiment. A pH change at some limiting CO_2 concentration is the method used to discriminate between CO_2 requirement for growth or a bicarbonate requirement. Substituting known CO_2 fixation products (permeable) for CO_2 and inducing alternate biochemical pathways which circumvent the need for a CO_2 fixation reaction, has been used to tentatively identify the growth regulatory system. This will be confirmed by analyzing the cells after pulse labeling experiments. At present, E. coli growing anaerobically appears to be the best choice of a model system because endogenous production of CO_2 is slow and the CO_2 can be effectively removed. After the identity of the CO_2 regulatory system is confirmed by labeling experiments, conventional biochemical procedures will be used to attempt to determine the interrelationship between this system and those related to cell growth, i.e. initiation of DNA replication and protein synthesis.

Major Findings:

Physiological studies on the effect of CO_2 on E. coli growing aerobically were completed, those on E. coli growing anaerobically are nearing completing, and the effects of CO_2 on normal (WI-38) and transformed (WI-18) mammalian cell lines were begun. In each instance, CO_2 was required to initiate growth, growth once initiated continued to depend on CO_2, and the rate of growth was controlled by the concentration of CO_2. Regulation of E. coli growth by CO_2 has interesting characteristics. Without CO_2, cells do not die (as in thymidine-less death); nor is there any evidence for continued DNA replication or protein synthesis which should product synchrony of growth when CO_2 was resupplied. It is as if CO_2 is a master switch which suspends growth related activities until CO_2 is available again to reinitiate those processes. The accepted role of CO_2 in heterotrophic fixation is to provide C_4 dicarboxylic acid for the tricarboxylic acid cycle and for synthesis of aspartic acid. Though E. coli is permeable

to aspartic acid, the CO_2 requirement was not relieved by substituting this or other products into which CO_2 is fixed. Malonate, however, was 67% as effective as CO_2, suggesting CO_2 exerts its effect by controlling fatty acid synthesis. If this proves to be the site of CO_2 regulation, it opens exciting possibilities for the study and control of growth. Fatty acids are primary constituents of cell membranes.

Tissue culture experiments have shown that CO_2 is as critical a nutrient for tissue as for bacteria. The optimum concentration spans a narrow range and is easily missed unless relatively small incremental differences in CO_2 concentration (as well as O_2 concentration) are tested. Two cell lines derived from similar tissue were selected for comparison. Normal cells required less oxygen and considerably more CO_2 than the transformed counterpart. By using the optimal CO_2 and O_2 concentrations derived from these studies, we have successfully cloned WI-38 through two successive cloning procedures. We intend to determine if these CO_2 and O_2 concentrations are sufficient to extend the normal life expectancy of WI-38 beyond 50 generations.

Significance to Biomedical Research:

The diversity of cells whose growth is similarly affected by CO_2 (bicarbonate) indicates a fundamental metabolic role for CO_2. The significance of this role is paramount because it controls growth. Unlike other required substrates which cause either unbalanced growth, 'cell aging' or cell death when they are selectively depleted, CO_2 deletion causes none of these. The immediate growth response to CO_2 suggests a key metabolic function is controlled, which, in turn, controls other systems. It is particularly intriguing that the CO_2 effect might be exerted in the fatty acid synthesizing system. Fatty acids are major components of cell membranes. An interrelationship of membrane synthesis, DNA replication and protein synthesis in whole cells has been repeatedly suggested in work of others, but no promising approach has been found for independently controlling one of the systems.

A more immediate potential application of these results is in tissue culture. Primary isolates of transformed cells are difficult to achieve because transformed cells are overgrown by normal cells. Our early results with only two cell lines indicate sufficient differences in optimum O_2 and CO_2 concentrations exist to provide selective pressure favoring growth of either cell type in a mixed population. This could prove to be a diagnostically useful procedure for isolating and identifying tumor cells. The same procedure may be useful in establishing heretofore difficult tissue cultures.

Publications:

None

LABORATORY OF CLINICAL INVESTIGATION

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Summary of Program
Laboratory of Clinical Investigation
July 1, 1975 - June 30, 1976

Sheldon M. Wolff, M. D., Chief of Laboratory
and Clinical Director, NIAID

Introduction

The Laboratory of Clinical Investigation has continued to maintain a national leadership position in clinical research in the areas of allergy, clinical immunology and infectious diseases. Recognition, in all forms, of this position continues to come to the staff.

Research Accomplishments

IMMUNOLOGIC RECONSTITUTION

Earlier studies had shown that patients with candidiasis that began during infancy or early childhood had congenital immunologic defects. These findings did not explain the occurrence of a clinically identical syndrome in older subjects. Recent experiments have shown an active suppressor substance (a cell or a cell product) in some of these patients. (Kirkpatrick, Sohnle, Chapman, and Smith)

The study of transfer factor in the treatment of candidiasis is continuing. Some patients have been in remission for over five years. In an attempt to assess the importance of the specificity of the transfer factor donors, candidiasis patients are now being treated with transfer factor from donors who lack cellular immunity to candida. (Chapman, Hoffman, Smith and Kirkpatrick)

Skin biopsies from nine candidiasis patients have been studied by immunofluorescent microscopy; five specimens contained C3, three of these contained properdin, only one contained candida antigen. C4 and immunoglobulins were not found in any specimen. The data suggested that candida antigens activated the complement system via the alternative pathway, thereby generating inflammatory mediators. In vitro experiments showed that candida extracts did cause production of chemotactic factors by this mechanism. (Sohnle and Kirkpatrick)

These experiments have been extended to two guinea pig models. In one, infections are produced under occlusive dressings. Clearance of organisms from these lesions involves complement activation and neutrophil infiltration, but is independent of the immune status of the animal. The second model does not use occlusive dressings. Clearance of organisms from these lesions involves increased epidermal growth, scale formation and sloughing of the infection. This response is related to delayed hypersensitivity to candida. (Sohnle and Kirkpatrick)

Progress toward characterization of transfer factor continues. A major component responsible for absorbance at 255 nm was shown to be hypoxanthine. Subsequent experiments have indicated that hypoxanthine is not essential for

the activity of transfer factor. Current efforts are directed at fractionation of transfer factor by ion exchange chromatography and establishment of test systems in animals. (Chapman, Hoffman, Robinson, Smith, and Kirkpatrick)

ALLERGIC DISEASES

The Allergic Diseases Section studies the mediators and biochemical pathways which underlie allergic reactions. The proteins required for the generation of bradykinin, namely, Hageman factor, prekallikrein and high molecular weight (HMW) kininogen were found to be required for the surface dependent initiation of coagulation and fibrinolysis. The function of the active site of bound Hageman factor was found to be augmented in a stoichiometric fashion by HMW kininogen. This converts sufficient prekallikrein to kallikrein to initiate a positive feedback enzymatic activation of Hageman factor by kallikrein. HMW kininogen also facilitates this interaction. Further, prekallikrein and HMW kininogen were found to circulate in plasma as a complex. Activated Hageman factor is normally inhibited by $C\bar{1}$ INH and to a lesser degree by antithrombin III. In the presence of heparin, antithrombin III binds and activates activated Hageman factor virtually instantaneously. (Kaplan, Meier, and Mandle)

In studies of the Hageman factor dependent fibrinolytic pathway, the plasminogen activator was found to cleave plasminogen to convert a single chain structure to a two-chain, disulfide-linked plasmin consisting of a heavy chain of molecular weight 56,000 and a light chain of 28,000. By incorporation of P^{32}DFP, the active site of plasmin was shown to be contained in the light chain. No binding of plasminogen activator with plasminogen was evident, thus the mechanism of activation appeared to be analogous to that observed with urokinase rather than streptokinase. (Kaplan and Yecies)

Histamine was found to enhance eosinophil migration to other chemotactic agents when tested at 10^{-6}M while at 10^{-5}M histamine generally inhibited eosinophil migration. The enhancement was mediated by H-1 receptors and the inhibition by H-2 receptors. The latter effect appeared to be secondary to the ability of high concentrations of histamine to raise the intracellular level of cyclic AMP. The chemotactic activity of histamine itself was not inhibited by H-1 or H-2 receptor antagonists. Thus, histamine may modulate eosinophil migration to other chemotactic agents as well as stimulating migration itself. Eosinophils appear to possess H-1 and H-2 receptors and may also possess a new kind of histamine receptor that has not previously been described. (Kaplan, Clark, and Gallin)

New methods for assessment of mediator release in the skin of patients with urticaria have been developed. It has been shown that patients with cold urticaria release histamine within the upper dermis when an ice cube is placed on their forearm. Thus, previous studies were confirmed demonstrating that histamine can be found in the venous circulation draining the extremity of patients whose hand was submerged in ice water. This technique is now being applied to acute and chronic urticarias of diverse etiologies. (Kaplan and Kaliner)

The biochemical events governing the immunologically-induced secretion of various chemical mediators of immediate hypersensitivity from human lung tissue and rat mast cells have been examined. A role for agents capable of increasing cyclic GMP in augmenting the secretion of mediators has been defined. Acetylcholine and $PGF_{2\alpha}$ (both hormones which are induced or secreted after allergic reactions) have been found to be potent agents for enhancement of mediator release and increases in cyclic GMP. A possible role for increased intracellular cyclic GMP as a modulator of allergic events is suggested. (Kaliner)

The role of various microorganelles in mediator secretion has also been examined and the mechanism whereby colchicine may prevent disaggregated microtubule function examined in both the rat mast cell and human lung tissue. (Kaliner)

The role of the eosinophil in parasitic diseases is being studied employing schistosomiasis as a model. An important role of eosinophils in attacking immobilized schistosoma antigen has been uncovered and is now being examined. Furthermore, the augmentation of this phenomena with activated complement byproducts is being investigated. (Kaliner, Metcalfe, and Ottesen)

CELLULAR IMMUNOLOGY

Based on the results of studies obtained in the Biologic Structure Section, models for the role of the macrophage in genetic control of T-cell responses to soluble protein antigens must consider: 1) an antigen specific receptor on the T cell; 2) cellular interaction structures identical to or closely linked to the alloantigens; 3) the IR gene product functioning either as the specific T-cell receptor or as an auxillary antigen recognition molecule on the macrophage and/or T cell; 4) antigen bound to metabilocally intact macrophages in a form resistant to proteolytic treatment and located in a cellular site not available to specific antibody; and 5) a macrophage associated nonspecific membrane trigger site or secretory product as suggested by the activation of aldehyde treated lymphocytes by normal macrophages. (Rosenwasser and Rosenthal)

Genetic control of immune responsiveness to both synthetic and natural polypeptide antigens has been found in experimental animals. Unfortunately, absolute demonstration of such phenomenon in man has not been possible because of the limited number of ethical antigens which can be used in man and their lack of defined physical structure. One antigen which might be of significant potential in such studies is insulin. Beginning with studies in inbred Strain 2 and 13, LCI scientists have found that cellular and humoral immune responsiveness of distinct regions of the molecule are under control of histocompatibility-linked genes. Strain 2 guinea pig T cells (as measured by proliferation or T cell help) recognize a determinant consisting of amino acids 6-11 of the A chain (α loop) while Strain 13 guinea pig T cells recognize a region in B chain. Nonetheless, the determinants recognized by B cells on both strains generally recognize shared determinants. Subsequently, studies were initiated in insulin requiring diabetics without obvious immunologic complications. Initial findings in ten patients who are diabetics requiring daily insulin did not exhibit in vitro antigen-induced T-cell proliferation.

At this time, it is unclear whether this is a "true" non-responsiveness or tolerization due to the mode of daily injection, i.e., development of suppressor cells, sequestration or high-dose tolerance. Studies are in progress to explore this. (Barcinski and Rosenthal)

STUDIES OF COMPLEMENT ABNORMALITIES IN MAN

This year, major emphasis has been placed upon the development of new therapy for patients with hereditary angioedema. A drug was sought which would be relatively nontoxic, which could be given to both men and women and which would control symptoms of the disease. For a number of reasons, a drug named Danazol produced by Sterling Winthrop Laboratories was chosen for study. A double-blind study was completed on the use of this drug in both men and women. The drug was shown to be highly effective in the treatment of hereditary angioedema. Of equal interest is the fact that the drug was shown to correct the genetically controlled biochemical abnormality, returning C1 esterase inhibitor and C4 levels to normal in most patients on therapy. It would appear that this drug will be the most useful available method for the treatment of this disease. A second project was begun during this period concerning the use of purified preparations of the C1 esterase inhibitor prepared by the American Red Cross for infusion therapy of patients with hereditary angioedema during acute attacks. These studies are only starting at the present time, and it is not yet known whether this will be effective therapy or not. (Frank, Gelfand, Alling [OSD, NIAID, NIH], and Gadek)

PATHOPHYSIOLOGY OF HEMOLYTIC ANEMIA

In the past year, studies of the pathophysiology of autoimmune hemolytic anemia have led to the following findings.

Studies of cold agglutinins have been completed and confirm some earlier observations made with IgM isoagglutinin antibodies. The clearance pattern of IgM-sensitized cells is entirely due to the characteristics of blood flow and the activity of cells of the reticuloendothelial system. These studies have shown that complement activation procedes almost instantaneously.

C3d-coated cells do not get cleared from the circulation if IgM is the antibody responsible for their attachment.

There are a limited number of sites that C3 can attach to the cells when the C3 is activated by cold agglutinin antibodies, and these sites differ for cold agglutinins and isoagglutinins. Cells with these sites occupied by C3d, the nonhemolytic fragment of C3, are less susceptible to lysis than normal cells. Rh antibodies were also shown to require the classical complement pathway for optimal clearance. In this case, the fragment responsible for clearance of the cells was shown to be C3d. Thus, C3d cooperates with IgG antibody but not with IgM to produce clearance. Initial studies using the techniques developed in this program in examining other diseases have been completed and an immunospecific defect in the clearance of particles from the circulation has been shown in patients with primary biliary cirrhosis. (Frank and Jaffe)

STUDIES ON THE INTERACTION OF ANTIBODY AND COMPLEMENT ON THE PRODUCTION OF IMMUNE DAMAGE

Many studies have come together in this project over the past year and have led to interesting and fruitful results. Among these are the following. It has been possible to show that guinea pigs genetically deficient in the fourth component of complement, originally discovered in our laboratories, show linkage between the gene controlling C4 synthesis and histocompatibility type. Presumably, the genes controlling these two separate synthetic steps are located on the same chromosome in the guinea pig as they have been shown to be in man. Demonstration of this provides the first animal model of linkage between a complement component in an animal and histocompatibility type. Thus, these animals now provide the only animal model for studies of complement synthesis and histocompatibility function. Studies have also been completed of the ability of myeloma proteins to activate the recently described alternative complement pathway. It has been possible to demonstrate that immunoglobulins of the IgM class and gamma 1 and gamma 3 sublcasses of IgG activate the alternative pathway. This was previously thought not to be the case. It was shown that at least half of the IgA myeloma proteins studied did not activate the alternative pathway. The single IgE myeloma protein studied activated the alternative pathway. Thus, some support is being developed for the concept that immunoglobulins can activate this pathway and lead to th duction of immune complex disease, a very important problem in man. Also, this year, studies were continued using the IgM and IgG complement-sensitized red cell reagents to characterize and identify various kinds of lymphoid tumors in man. This whole approach has turned out to be of great importance and is leading to a thorough reclassification of human malignancies in collaboration with the National Cancer Institute. A great many studies have been reported. Finally, the importance of the recently described complement receptor located in the human renal glomerulus has been demonstrated by an important series of studies examining biopsy specimens from patients with various kinds of renal disease. It was shown that patients who have immunoglobulin and complement deposition in the glomeruli in vitro, especially when this deposition is in a sbendothelial location in the glomerulus, have loss of their glomerulose complement receptor activity; therefore, it would appear that this receptor plays an important role in deposition of complement components in the glomeruli. (Frank and Gaither)

CLINICAL MYCOLOGY

The multicenter collaborative clinical comparison of two different drug regimens for cryptococcal meningitis has been completed. The study, which was organized and coordinated by the Clinical Mycology Section, found convincing proof that a new combination 5-fluorocytosine-amphotericin B regimen, given for only six weeks, was at least as good as the older ten-week amphotericin B regimen. (Bennett)

Another study refuted the prior notion that 5-fluorocytosine interferes with fungal RNA and instead showed clearly that DNA synthesis is blocked by inactivation of the enzyme, thymidylate synthetase. This knowledge may prove useful in choosing companion drugs that may enhance the chemotherapeutic effect of 5-fluorocytosine. (Bennett and Diasio [LCP, NCI, NIH])

CRYPTOCOCCUS NEOFORMANS

Study revealed the sexual reproduction cycle of Cryptococcus neoformans. The isolates of C. neoformans are heterothallic and produce two different sexual states, designated as Filobasidiella neoformans and F. bacillispora.

Isolates of Cryptococcus neoformans producing the F. neoformans state were found to belong to serotypes A and D while the isolates producing F. bacillispora belong to serotypes B and C. Ultrastructure of hyphal septa and the mechanism of sexual sporulation in F. neoformans indicated that the fungus is a transitional form between lower and higher basidiomycetes. (Kwon-Chung)

PARASITIC DISEASES

Studies of cell-mediated immunity in patients with helminthic infections have been extended to schistosomiasis. Although it is too early to generalize, it seems that the same patterns of cellular immune response may occur in several of the chronic worm infections with the parasite living in the tissues. Individuals in the South Pacific living in the endemic area of filariasis but without microfilaremia exhibited strong cellular immune responses to filarial antigens while those with microfilaremia were poorly responsive. This defect was specific for filarial antigens (Ottesen). Cases of schistosomiasis studied within three months of initial infection with S. mansoni generated brisk in vitro cellular immune responses whereas lymphocytes from those with chronic infection had a diminished response to the same schistosome antigens (Ottesen and Neva). Thus, with both of these parasitic infections, a perfectly good cellular host response occurs initially but appears to diminish or be much less prominent with time, even though the parasite remains. This pattern of response may have important implications in the pathogenesis of parasitic infections.

HERPES VIRUS INFECTION

Examination of host responses in patients with recurrent mucocutaneous herpes simplex infections (types 1 and 2) of the oro-facial and genital areas, indicated that these infections recurred in the presence of high levels of circulating antibody. Cell-mediated immunity, as measured by a direct lymphocytotoxic assay against acutely infected target cells, is present at levels equal to or greater than those found in matched, sero-positive controls without recurrences. The presence of acute lesions is associated with significantly higher levels of cytotoxicity. Chronically infected target cells elicited different cytotoxic responses than did acutely infected cells. (Dolin and Reichman)

Final analysis of the nationwide trial of ara-A in herpes zoster infections, including patients from this unit, indicates that the compound speeds resolution of cutaneous lesions. Additional trials of ara-A in other herpetic infections are continuing. Examination of in vitro effect of ara-A against Epstein-Barr virus suggests that virus-specific DNA synthesis in inhibited. (Dolin, Reichman, and Coker-Vann)

VIRAL GASTROINTESTINAL AND RESPIRATORY TRACT INFECTIONS

Experimental influenza infections in man resulted in the development of virus-specific cell-mediated immune responses, as measured by blastogenesis of peripheral lymphocyte cultures stimulated with homologous, purified, inactivated virus. This response was correlated with the development of Hemagglutinin-inhibiting but not neuraminidase-inhibiting antibody. An assay of direct lymphocytotoxicity against virus-infected tissue culture cells was established, and levels of cytotoxicity were detected in lymphocytes from human, as well as experimental animal (mouse) infections. In the latter system, the H2 histocompatibility locus appeared to be partially linked to cytotoxic capability. Human lymphocytes appear to have a hemagglutinin receptor for influenza virus. A new interferon inducer (poly ICLC) was administered intranasally to volunteers without local or systemic toxicity. However, in experimental challenge with rhinovirus 13, prophylactic and therapeutic intranasal administration of PICLC failed to provide protection from illness. Additional studies with higher doses of this and other antiviral compounds are planned. (Dolin and Reichman)

Intestinal secretions from volunteers experimentally infected with the parvovirus-like agents of viral gastroenteritis demonstrated immune aggregation of the homologous viral particles, consistent with the development of local IgG and IgA responses to viral infection. (Dolin)

In vitro studies with human parvoviruses (AAV) demonstrated that tissue culture cells chronically infected with herpes simplex virus or transformed by adenovirus potentiated AAV. This potentiating or "helper" function is currently under study. (Dolin)

IMMUNOSUPPRESSION

Leukocyte subpopulations have been isolated, identified and functionally characterized allowing correlation of specific subpopulations of cells involved in certain inflammatory and immunologically mediated diseases. Studies of the specific effects of immunosuppressive agents, particularly corticosteroids, have delineated the differential sensitivity of these subpopulations of cells to therapeutic intervention. In particular, corticosteroid administration affects certain subpopulations of thymus-derived (T) lymphocytes by an alteration of their distribution or circulation patterns while other subpopulations are suppressed in various functional capabilities. On the other hand, populations of non-T cells which mediate cytotoxic killer cell function against various tissue targets are relatively resistant in their functional capabilities to the administration of corticosteroids. Corticosteroids affect these subsets of cells by impeding their availability or access to the inflammatory site. These studies have clear-cut clinical relevance in inflammatory and autoimmune diseases, organ transplantation, and tumor surveillance. Such studies have also allowed for a scientific basis for the design of corticosteroid treatment regimens which are effective in the suppression of disease activity with a minimum of compromise of host defense mechanisms against infections. (Fauci, Parrillo, Balow, and Hunninghake)

Studies of the distribution and functional properties of lymphocyte subpopulations in normal human bone marrow and in the bone marrow of various inflammatory disease states have added to our understanding of such phenomena in certain diseases as well as during various chemotherapeutic regimens. In addition, the demonstration of mature T cell function as well as functionally mature cytotoxic effector cells in the normal human bone marrow have broadened understanding of the possible mechanisms of the graft-versus-host reaction seen in bone marrow transplantation. (Fauci)

Heterogeneous subpopulations of eosinophils in the idiopathic hypereosinophilic syndrome have been identified and their functional capabilities characterized. This has led to an understanding of the mechanisms whereby eosinophils infiltrate organs and result in tissue damage. The differential sensitivity of these eosinophil populations to corticosteroid administration has been described allowing greater specificity in the choice of therapeutic regimens. (Parrillo and Fauci)

A highly reproducible hemolysis-in-gel plaque forming cell assay for the measurement of single cell antibody production following primary in vitro activation of human bone marrow-derived (B) lymphocytes from tonsil, bone marrow, spleen and peripheral blood has been developed. Such a model provides the means for indepth probing of the complex mechanisms of activation and suppression of human B lymphocytes towards antibody production in the normal immune response as well as in disease states characterized by an over or under production of antibody. In addition, the specific effects of various immunosuppressive agents on the events associated with B cell activation in various antibody-mediated diseases can be monitored. (Fauci and Haynes)

MODULATION OF THE INFLAMMATORY RESPONSE

Interaction of leukocytes with chemotactic factors results in decreases in cell surface charge; this decrease in surface charge is blocked by steroids but not colchicine or cytochalasin B. Electrophysiological studies on the interaction of cultivated human macrophages with the chemotactic factors in endotoxin-activated serum demonstrated that the activated serum induced a rapid hyperpolarization of macrophage membranes which preceded cell contraction and pseudopod formation. Related studies demonstrated the requirement of calcium and magnesium for leukocyte migration. In studies of calcium exchanges, it was shown that interaction of leukocytes with chemoattractants resulted in a net calcium release and inhibition of calcium uptake. Shifts in intracellular calcium stimulated by the calcium ionophore A23187 were associated with modulation of cyclic nucleotide metabolism with increases in cAMP by A23187. Other agents such as carbachol, serotonin and ascorbic acid, which increased cGMP, amplified leukocyte migration. (Gallin)

Specific orientation of neutrophil centrioles, microtubules and submembranous microfilaments was demonstrated during chemotaxis. Studies with colchicine and cytochalasin B demonstrated that migration requires microfilaments whereas direction appears dependent on microtubule assembly. In addition, PMN granules orient in the leading front of migrating PMN's. (Gallin and Malech [LCB, NCI, NIH])

A preferential release of specific granules from PMN's was demonstrated during migration in vitro and exudation in vivo. Specific granules contain an enzyme which can activate the complement cascade to produce the chemotactic factor C5a. Azurophil granules, which are released late after phagocytosis, contain an enzyme which inactivates the chemotactic activity of C5a. (Wright and Gallin)

Levamisole increased neutrophil and monocyte directed but not random migration in association with increases in cGMP. Four patients with defective leukocyte chemotaxis, high IgE and recurrent pyogenic infections were given levamisole and in each case significant improvement of in vitro chemotaxis was demonstrated after in vivo levamisole. (Wright, Kirkpatrick and Gallin)

PATHOGENESIS OF FEVER

An anti-human leukocytic pyrogen antibody has been prepared in rabbits. The material was shown to be specific for human leukocytic pyrogen and to block its biologic activity. When attached to sepharose and used as an immunoabsorbent, it has proven to be a powerful tool for the preparation of leukocytic pyrogen with greater purity than ever before obtained. (Dinarello and Wolff)

NONSPECIFIC RESISTANCE TO INFECTION

Materials obtained from the Pasteur Institute have been the subject of intensive investigation. These compounds, primarily of microbiologic origin, are almost non-toxic and are potent adjuvants. It has been found that they possess the ability to induce nonspecific resistance to a wide variety of experimental infections. Clinical trials of these compounds are being planned. (Elin [CPD, CC, NIH] and Wolff)

OBSERVATIONS OF SECRETORY AND CELLULAR IMMUNITY IN THE RESPIRATORY AND GASTROINTESTINAL TRACTS

The general area of pulmonary host defenses and nosocomial infections is being investigated, using Pseudomonas pneumonia as the prototype infection because of its frequent occurrence. Initial work, done in rabbits, has been expanded to humans, to explore ways of manipulating antibody production in the respiratory tract and to assess the effectiveness of these antibodies in promoting phagocytosis and killing by alveolar macrophages. IgG was found to be a superior opsonin to IgA in the lung; IgM was ineffective. This specific opsonic activity was correlated and explained by additional studies, which identified surface membrane receptors on alveolar macrophages. The macrophages have receptors for IgG and for the third component of complement. As yet, the importance of the complement receptor (C_3G) is uncertain because complement activity is generally low in broncho-alveolar lavage fluid. Evidence would suggest that the complement system might augment the opsonic activity of IgG antibody in lung secretions. (Reynolds, Kazmierowski, and Frank)

The ultrastructure of canine secretory IgA, secretory component and J-chain, purified from colostrum, is being investigated to elucidate the assembly of the secretory IgA molecule. Work this year featured chemical and structural analyses of secretory component, which show it to be similar to those reported for the human, rabbit and cow, and work pertaining to the binding sites between fragments of the alpha chain of secretory IgA and secretory component. (Thompson and Reynolds)

Studies pertaining to the development of cellular immunity in the gastrointestinal tract utilized a rat model of Trichinella spiralis gut infection. It was shown that T-lymphocytes in Peyer's patch lymphoid tissue did not acquire antigen-induced reactivity upon initial contact but later when other systemic lymphoid tissue had acquired cellular reactivity. Thus, the primary role of lymphocytes in Peyer's patches has not yet been elucidated. (Reynolds)

Administrative, Organizational, Other Changes

As noted in last year's report, some personnel additions were to occur. The changes have taken place and the LCI, manpower-wise, is at its highest level in recent years. Dr. John Gallin returned last July, and his laboratory is now responsible for functional and physiological studies of white blood cells and their defects. Dr. Michael Kaliner arrived during the summer and has initiated studies of mediators of the allergic reaction. Dr. Charles Dinarello returned in September and has devoted his attention to studies of the purification of human leukocytic pyrogen. Unfortunately, Dr. John Atkinson has decided not to return to the NIH. He has accepted a position as Head of the Rheumatology Division in the Department of Medicine at Washington University. It is with sadness that I must report that Dr. Herbert Y. Reynolds will be leaving the LCI in August to become Head of the Pulmonary Diseases Division at the Yale University School of Medicine. The need for additional support personnel and more space for existing staff necessitates that Dr. Reynolds not be replaced at this time.

Honors, Awards, and Scientific Recognition

Many forms of recognition continue to accrue to the members of the LCI. Our senior staff continues to increase its participation in important areas both on the local and the national scenes. For example, Dr. Rosenthal presented a course in Brazil, Dr. Kaplan gave lectures in Germany, Dr. Wolff lectured in Turkey and was a Visiting Professor in Brazil. Dr. Wolff served as a member of the American delegation to a CENTO conference in Turkey. A book entitled, Immunologic and Infectious Reactions in the Lung, edited by Drs. Kirkpatrick and Reynolds, appeared this year. Eight members of the LCI have been elected to membership in the American Society for Clinical Investigation. Dr. Fauci was elected a councillor of the Eastern Section of the American Federation for Clinical Research. Dr. Wolff was elected to a three-year term as a councillor of the Infectious Diseases Society of America during this past year. Dr. Frank continues to direct the NIH clinical clerkship in immunology program, and Dr. Bennett is in charge of the program in infectious diseases. Dr. Reynolds served as Chairman of the NIH Clinical Research Committee and Dr. Dolin as Chairman of the NIAID Clinical Research Committee.

The members of the LCI have received many other forms of recognition which are too numerous to mention. The above merely highlights the extent and quality of the recognition received by members of the laboratory.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00042-11 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Fever and Host Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Wolff	Chief, Lab. of Clin. Invest.	LCI	NIAID
OTHER:	C. A. Dinarello	Guest Worker	LCI	NIAID
	A. S. Fauci	Head, Clin. Physiol. Section	LCI	NIAID
	J. I. Gallin	Senior Investigator	LCI	NIAID
	J. E. Gadek	Clinical Associate	LCI	NIAID
	D. G. Wright	Clinical Associate	LCI	NIAID
	J. B. Edelin	Biol. Lab. Tech.	LCI	NIAID
	L. Renfer	Chemist	LCI	NIAID

COOPERATING UNITS (if any) D. W. Alling (OSD, NIAID, NIH); W. Barth (Wash. Hosp. Center, Wash., D. C.); E. Besa (Presbyterian-Univ. of Penna. Med. Center, Philadelphia, Pa.); L. Chedid (Pasteur Institute, Paris, France); J. L. Costa (LCS, NIMH, NIH);
(Continued below)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4-8/12	2-8/12	2

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms underlying the pathogenesis of fever in man are a major area of interest. Present investigations are directed at: (1) purification of human leukocytic pyrogen; and (2) development of a radioimmunoassay for human leukocytic pyrogen.

Receiving equal attention and effort are some of the mechanisms responsible for nonspecific resistance to infection. Inducers of resistance such as bacterial endotoxins, other bacterial products and synthetic adjuvants are under study.

Cooperating Units (Con't.): D. C. Dale (Dep't. of Med., Univ. of Wash. Hosp., Seattle, Wash.); R. J. Elin (CPD, CC, NIH); C. Finch (Dep't. of Hematology, Univ. of Wash. Hosp., Seattle, Wash.); F. Gardner (Dep't. Med., Univ. of Texas, Galveston, Texas); H. R. Gralnick (Hematology Service, CPD, CC, NIH); G. A. Padgett (Wash. State Univ., Pullman, Wash.); C. W. Parker (Dep't. Internal Med., Wash. Univ. School of Med., St. Louis, Mo.); M. Perlow (IRP, NINCDS, NIH); S. Leapman (National Naval Medical Center, Bethesda, Md.)

Project Description:

Selected patients are being studied on the wards and in the laboratory. The patients can be divided into the following groups: patients with a) recurrent fever; b) Chediak-Higashi syndrome; c) granulomatous disorders; d) cyclic neutropenia; e) eosinophilia; f) recurrent infections; g) familial Mediterranean fever; h) aplastic anemia; and i) normal volunteers.

Extensive studies are in progress relating to the pathogenesis of fever, nonspecific resistance to infection, immune and other responses of humans and experimental animals to infections, immunosuppression, etc.

Objectives:

1. To assess varied biologic responses of normal and a variety of patients to pyrogens.

2. To intensively study groups of patients with such poorly defined illnesses as familial Mediterranean fever, recurrent fever, Chediak-Higashi syndrome, midline granuloma, Wegener's granulomatosis, and cyclic and other neutropenias in an attempt to better delineate the clinical syndrome.

3. To study the mechanisms responsible for the release of endogenous pyrogen.

4. To characterize and purify leukocytic pyrogen.

5. To assess the effect of immunosuppressive agents on human diseases.

6. To explore the pathogenesis of the increased susceptibility to infection in a variety of diseases.

7. To define the features of the beige mouse which are similar to the Chediak-Higashi syndrome of man.

8. To determine the susceptibility of beige mice to infection.

9. To assess the protective effects of endotoxin and other microbial and synthetic products in mice challenged with various microorganisms and, if possible, to characterize the humoral and cellular factor(s) responsible for protection.

10. To evaluate the specificity of limulus amebocyte lysate for compounds other than endotoxin.

11. To evaluate the utility of the limulus amebocyte lysate test to detect endotoxemia in man.

12. To study the effects of oral and parenteral corticosteroids in the syndrome of cyclic neutropenia.

Methods Employed:

Patients and volunteers receive varying doses of endotoxin and etiocholanolone. Rectal temperatures are taken and a variety of responses are determined: antibody levels, immunoglobulins, plasma cortisol, growth hormone, serum iron, clotting factors, etc.

Miceoorganisms were grown in appropriate media, quantitated by turbidity and intravenously injected into mice.

Limulus amebocyte lysate was prepared from limulus crabs. The lysate is used to study endotoxemia in man and specificity of the reaction.

Mice are given endotoxin, bacterial or synthetic materials and subsequently challenged at varying time intervals with such infectious agents as Candida albicans, Plasmodium berghei, etc.

Human peripheral leukocytes are incubated overnight with killed S. albus organisms. The resulting supernatant contains leukocytic pyrogen. This material is concentrated by means of alcohol precipitation, dialysis, column chromatography and fractionation, isoelectric focusing and electrophoresis. Antibody to this material is prepared in rabbits.

Patient Material:

Patients are admitted to the Clinical Center, as are normal volunteers, for these studies. In addition, patients from other Institutes participate in these studies.

Major Findings:

Human leukocytic pyrogen has been found to be at least two molecules, one from polymorphonuclear leukocytes and the other from monocytes. Antibodies to human leukocytic pyrogen have been prepared in rabbits. Through the use of these antibodies, immunoabsorbent columns have been prepared allowing considerable purification of this material.

Cyclophosphamide has produced striking remissions and clinical improvement in patients with Wegener's granulomatosis, nephrosis and a variety of hypersensitivity diseases.

The limulus amebocyte lysate test has limited clinical utility for the detection of endotoxemia or Gram-negative rod septicemia in man.

Neo-WSA, a delipidated material derived from Mycobacteria smegmatis, induces nonspecific resistance to infection in mice and the mechanism differs

from endotoxin, the classical compound for the induction of nonspecific resistance. Other bacterial and synthetic compounds have been found to have similar properties.

The platelets of patients with the Chediak-Higashi syndrome have been found to have a markedly reduced number of granules and very low levels of serotonin.

Preliminary studies suggest that etiocholanolone may be a useful agent in the therapy of aplastic and hypoplastic anemia.

Colchicine can be employed as a therapy of the acute attacks of familial Mediterranean fever in addition to its prophylactic value.

Kidney transplants have been successfully done in two patients with renal failure secondary to amyloidosis from familial Mediterranean fever.

Significance to Biomedical Research and the Program of the Institute:

The large number and wide variety of studies listed above provide considerable new knowledge dealing with host response mechanisms to a wide variety of clinical and experimental diseases and the pathogenesis of fever.

Proposed Course:

The above outlined studies are to be continued and some areas are to be expanded.

Publications:

1. Hawiger, J. and Wolff, S. M.: The Shwartzman reaction. In Brent, L. and Holborow, J. (Eds.): Progress in Immunology II. Amsterdam, North-Holland Publishing Co., Vol. 4, 1974, pp. 359-362.

2. Wolff, S. M., Fauci, A. S., and Dale, D. C.: Unusual etiologies of fever and their evaluation. Ann. Rev. Med. 26: 277-281, 1975.

3. Brodey, P. A. and Wolff, S. M.: Radiographic changes in the sacroiliac joints in familial Mediterranean fever. Radiology 114: 331-334, 1975.

4. Perlow, M., Dinarello, C., and Wolff, S. M.: A primate model for the study of human fever. J. Infect. Dis. 132: 157-164, 1975.

5. Elin, R. J., Vesell, E. S., and Wolff, S. M.: Effects of etiocholanolone-induced fever on plasma antipyrine half-lives and metabolic clearance. Clin. Pharmacol. and Therapeut. 17: 447-457, 1975.

6. Kimball, H. R., Ford, G. H., and Wolff, S. M.: Lysosomal enzymes in normal and Chediak-Higashi blood leukocytes. J. Lab. Clin. Med. 86: 616-630, 1975.

7. Elin, R. J., Robinson, R. A., Levine, A. S., and Wolff, S. M.: Lack of clinical usefulness of the limulus test in the diagnosis of endotoxemia. New Engl. J. Med. 293: 521-524, 1975.

8. Elin, R. J. and Wolff, S. M.: Biology of endotoxin. Ann. Rev. Med. 27: 127-141, 1976.

9. Elin, R. J., Wolff, S. M., and Chedid, L.: Nonspecific resistance to infection in mice induced by a water-soluble adjuvant derived from Mycobacterium smegmatis. J. Infect. Dis. 133: 500-505, 1976.

10. Rudbach, J. A., Akiya, F. I., Elin, R. J., Hochstein, H. D., Luoma, M. D., Milner, E. C. B., Milner, K. C., and Thomas, K. R.: Preparation and properties of a national reference endotoxin. J. Clin. Micro. No. 1, 21-25, 1976.

11. Wolff, S. M., Claman, H., Schur, P., Gleich, G., Lieberman, P., and Stechschulte, D.: Allergy and clinical immunology: an annotated bibliography of recent literature. References to journal articles and other papers. Ann. Intern. Med. 84: 505-509, 1976.

12. Wolff, S. M.: Approach to the patient with fever. Proceedings of the CENTO Conference on the Use and Misuse of Antimicrobial Drugs. In press.

13. Elin, R. J. and Wolff, S. M.: Bacterial endotoxins. In CRC Handbook on Microbiology. Second edition. In press.

14. Tsan, M. F., Newman, B., Chusid, M. J., Wolff, S. M., and McIntyre, P. A.: Surface sulfhydryl groups and hexose monophosphate shunt activity in resting human polymorphonuclear leukocytes. Brit. J. Haematology. In press.

15. Dinarello, C. A. and Wolff, S. M.: Exogenous and endogenous pyrogens. In Brazier, A. B. (Ed.): Brain Dysfunction in Infantile Febrile Convulsions. Raven Press, Vol. 2. In press.

16. Elin, R. J. and Wolff, S. M.: Bacterial endotoxins. In Laskin, A. I. and Lechevalier, H. A. (Eds.): CRC Handbook of Microbiology. Cleveland, Ohio, CRC Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00043-11 LCI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunology and Chemotherapy of Systemic Mycoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	K. J. Kwon-Chung	Research Microbiologist	LCI NIAID

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LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3-6/12	1-6/12	2

SUMMARY OF WORK (200 words or less - underline keywords)

A multicenter collaborative randomized clinical comparison of 5-fluorocytosine - amphotericin B with amphotericin B in cryptococcal meningitis has found that six weeks of the combination drug is at least as good as ten weeks of the single drug. Mechanism of action of 5-fluorocytosine on Candida albicans was found to be inhibition of the enzyme, thymidylate synthetase. Serotype D Cryptococcus neoformans was found to be much more prevalent in Europe than in the U.S.A.

COOPERATING UNITS:

B. Fields (VA Little Rock Hosp.), M. Bradshaw (Methodist Hosp, Houston), T. Williams (Methodist Hosp, Houston), J. Warner (Ohio State U), G. Cobbs (U Ala in Birmingham), D. Alling (OSD, NIAID, NIH)

PHS-6040
(12-75)

Project Description:

1. The NIH-sponsored multicenter collaborative study of cryptococcal meningitis has concluded a two-year randomized, prospective comparison of the older, 10-week amphotericin B treatment regimen with the newer, 6-week combination of amphotericin B - 5-fluorocytosine. Of the 69 patients meeting the criteria for retention in the study, 63 received the protocol drugs within the defined limits, 33 receiving the older and 30 the newer regimen. Among these 63, there was no significant difference in therapeutic effect even though the newer regimen was 4 weeks shorter. The combination therapy experienced only 3 relapsed or failed patients and 4 patients died during therapy after day 7. With amphotericin B alone, 11 failed or relapsed and 3 died after day 7. Further, the rate of cultural conversion was significantly more rapid with the combination ($p<0.05$). The major limitation of the combination regimen was suspected or definite toxicity, which accounted for 8 out of 10 patients who were excluded from analysis because of inadherence to the combination drug regimen. Toxicity was not fatal and consisted of 5-fluorocytosine-induced leukopenia, thrombocytopenia or diarrhea. Toxicity also occurred due to amphotericin B but was similar on both regimens and did not cause protocol inadherence. Exclusion of protocol inadherent patients from analysis of chemotherapeutic effect did not bias the data, in that 4 out of 5 such patients randomized to amphotericin B and 8 of 10 randomized to the combination were cured or improved.

2. Mechanism of antifungal action of 5-fluorocytosine upon Candida albicans has been studied in collaboration with Dr. Diasio (NCI). Clear evidence was found that 5-fluorocytosine is converted to the active metabolite F-dUMP, a noncompetitive inhibitor of the enzyme thymidylate synthetase. This blocks DNA synthesis by interfering with the conversion of dUMP to thymidylate. Proof was obtained by showing that cytoplasm of Candida cells exposed to 5-fluorocytosine exhibited a prompt decrease in thymidylate synthetase activity, increase in the precursor, dUMP, and appearance of the inhibitor F-dUMP.

3. Serotyping of C. neoformans obtained from widely separated sites has been continued, in order to provide Dr. Kwon with isolates for her studies and to further elucidate the geographic distribution of serotypes. Two new findings were that serotype D was strikingly more common in isolates from Europe (85% of 27 isolates) than elsewhere (3% of 237 isolates) and that 78 isolates from 53 sites in nature were all types A or D. Thirty of these isolates were obtained from California where 37% of 51 patient isolates were types B or C. It is becoming clear that types B and C do not share the same natural habitats with types A and D. This discrepancy is in keeping with Dr. Kwon's discovery that types A and D constitute a different species than types B and C. Her discovery of haustoria-like appendages on types B and C suggests this species may be a plant pathogen, rather than a saprophytes of pigeon droppings.

4. A cell culture model for 5-fluorocytosine toxicity has been discovered. Drug concentrations comparable to those found in serum of patients with leukopenia have been found to decrease by more than 50% the mitogen-induced deoxyuridine incorporation in human lymphocytes. This should provide a useful

tool for determining the enzymatic blockage responsible for 5-fluorocytosine-induced leukopenia in man.

Proposed Course:

1. The collaborative study is no longer admitting patients but will continue to follow patients until 12 months post-treatment.

2. Studies on the mechanism of action of 5-fluorocytosine have been completed. Future studies will concern mechanism of toxicity and attempts to block 5-fluorocytosine-induced bone marrow suppression.

3. Studies on the geographic distribution of serotypes are being completed. Future studies on serotypes B and C will be to describe this as a biochemically distinct species and to try to locate its site in nature.

Publications:

1. Gelfand, J. A. and Bennett, J. E.: Active Histoplasma Meningitis of 22-years' duration. J. Am. Med. Assoc. 233: 1294-5, 1975.

2. Schimpff, S. C. and Bennett, J. E.: Abnormalities in cell-mediated immunity in patients with Cryptococcus neoformans infection. J. Allergy Clin. Immunol. 55: 430-441, 1975.

3. Aisner, J., Schimpff, S. C., Bennett, J. E., Young, V. M., and Wiernik, P. H.: Cluster of aspergillus infections in patients with cancer: an association with fire-proofing materials in a new hospital. J. Am. Med. Assoc. 235: 411-412, 1976.

4. Bennett, J. E.: Review of Amphotericin B. In Williams, J. D. and Geddes, A. M. (Eds.): Chemotherapy New York, Plenum Press, 1976, Vol. 6. In press.

5. Ellner, J. and Bennett, J. E.: Chronic meningitis. Medicine In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00044-09 LCI

PERIOD COVERED: July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Observations on Secretory Immunoglobulins (includes Humans, Dogs and Guinea Pigs)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Y. Reynolds, Head, Bacterial Diseases Section, LCI, NIAID

OTHER: J. A. Kazmierowski, Clinical Associate, LCI, NIAID

COOPERATING UNITS (if any)

M. J. Waxdal (LI, NIAID, NIH) Z01 AI 00038-04 LI
W. Strober (MB, NCI, NIH) Z01 CBD 4014-08 MET

LAB/BRANCH: Laboratory of Clinical Investigation

SECTION: Bacterial Diseases Section

INSTITUTE AND LOCATION: NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-4/12	4/12	1

SUMMARY OF WORK (200 words or less - underline keywords)

The ultrastructure of canine secretory component and J-chain are being investigated now that they have been isolated and purified from secretory IgA; the combination of these chains with 7S IgA monomer may elucidate the assembly of the secretory IgA molecule.

While investigating the origin of precursor cells that differentiate into immunoglobulin secreting plasma lymphocyte cells in the gastrointestinal tract, the interactions of orally administered antigens and processing cells (T-lymphocytes) in gut-associated lymphoid tissue (Peyer's patches) were studied in adult guinea pigs. A detailed identificatopm of lymphoid cell populations in Peyer's patches and an assessment of lymphocyte activity (afferent and efferent T-cell functions) were made. To further assess primary immune responses in the gastrointestine, a rat model of Trichinella spiralis gut infection was used to observe the sequence of developing cellular immunity in Peyer's patches and other lymphoid tissues. In this parasitic gut infection, the Peyer's patch lymphoid tissue did not appear to be the first site of cellular responsiveness but rather to acquire cellular reactivity only when other lymphoid elements in the infected host have also required similar antigen-induced reactivity.

Project Description:

Objectives:

1. To complete the molecular model for canine secretory IgA and identifying the assembly and interactions of secretory component (SC) and J-chain.

2. To elucidate the ultrastructure of secretory component and *in vitro* to study the susceptibility to proteolysis of dimeric serum and secretory IgA. Further, enzymes in gut epithelial cells (from *in vitro* cell cultures) responsible for synthesis of SC and for the assembly of SC and IgA are being sought.

3. To examine the gut associated lymphoid tissue (Peyer's patches) in dogs and guinea pigs for its response to bacterial antigens and for its capacity to process antigens and to produce immunoglobulins (antibody).

4. Protective effect of secretory antibodies (IgA and IgG) in experimental gastrointestinal infections (cholera and non-bacterial infectious diarrhea).

Methods Employed:

Gel filtration and ion exchange chromatography, affinity and immunoabsorbent chromatography, standard techniques of zonal electrophoresis, immunodiffusion, sedimentation velocity and equilibrium ultracentrifugation; equilibrium dialysis and fluorescence quenching for determination of binding energies; liquid scintillation spectroscopy; polyacrylamide disc gel electrophoresis; iso-electrofocusing; amino acid analysis; and amino acid sequence analysis. For intestinal lymphoid cell tissue cultures, autoradiography; immunofluorescence and stimulation of lymphocytes by mitogens including PHA, Con A, endotoxin *et cetera*, measuring ^{3}H-thymidine incorporation or lymphokine production (MIF).

Major Findings:

Secretory component, predominantly attached to colostral and intestinal IgA through covalent (disulfide) bonding, has been dissociated, and isolated and its molecular weight of approximately 70,000 daltons established. Further isolation of J-chain, using polyacrylamide and isoelectric focusing separation techniques, from canine colostrum and serum IgM has been done. Further characterization of SC with amino acid analysis and sequence was carried out.

Free secretory component (FSC) was isolated from canine colostrum, using a combination of immunoabsorbants, gel filtration and ion-exchange chromatography. The yield of FSC from one liter of colostrum was approximately 100 mg. With alkaline urea and sodium dodecyl sulfate (SDS) disc gel

electrophoresis, FSC migrated as a single band with a corresponding molecular weight (MW) of 77,500 daltons ± 6500. The carbohydrate content of FSC was 19% of weight (6% neutral hexose, 6.4% glucosamine and 6.6% galactosamine). The amino acid composition of FSC was similar to those reported for the human, rabbit and cow. The amino acid sequence for the 12 NH_2-terminal residues of FSC was:

Lys-Ser-Pro-Ile-Phe-Gly-Pro-Glu-Glu-Val-Asn-Ile.

The first 10 residues of this sequence were identical to that of human and bovine FSC. Canine FSC was further subjected to cyanogen bromide cleavage and the products were analyzed by SDS gel electrophoresis. Two bands were observed with estimated MW's of 55,000 daltons ± 5000 and 17,000 ± 1500. Amino acid analysis and SDS polyacrylamide gel electrophoresis revealed that only the larger fragment was composed of several smaller fragments linked by disulfide bonds. sIgA was stripped of non-covalently bound subunits by gel filtration in 6 M guanidine HCl and it was found that greater than 90% of bound secretory component (BSC) was covalently attached. Following cleavage, this stripped sIgA released J chain and light chain fragments as well as two large fragments of BSC, MW 55,000 d and 17,000 d. The larger fragment of BSC was similar to the corresponding fragment of FSC by amino acid composition, immunological reactivity, and MW. The J chain fragment was similar to the J chain obtained by reduction and alkylation of sIgA, as determined by amino acid composition, mobility during polyacrylamide gel electrophoresis and MW. These results suggest that in canine sIgA the major fragments of BSC and J chain are not mutually connected by disulfide bridges, but may be connected to different CNBr fragments of the α chain as previously shown for human sIgA.

An opportunity presented to investigate a patient with an apparent absence of secretory component in exocrine secretions which was in part responsible for his immunodeficiency disease. The observations have documented an unusual and rare clinical syndrome.

Peyer's patch lymphocytes have been isolated and successfully maintained in tissue culture from guinea pigs for periods exceeding seven days. These experiments faced a formidable technical problem considering the bacterial contamination present on the gut surfaces. The lymphocytes have been identified as approximately 33-55% B-type and 40% T-type. Further, the functional capacity of Peyer's patch lymphocytes have been eluciated. T-cells in Peyer's patches are capable of mounting a graft vs. host reaction to allogeneic cells and of responding to similar cell surface antigens in two way mixed lymphocyte reactions; in addition, these Peyer's patch cells can be stimulated to liberate a lymphokine which induces monocyte chemotaxis. Thus, Peyer's patch cells have been shown to have efferent and afferent functions identified with cell mediated immunity and to behave in a manner similar to other peripheral lymphoid tissue. To test the functional capacity of these cells, we used a rat model of Trichinella spiralis gut infection to observe the sequence of developing cellular immunity in Peyer's patches and other lymphoid tissues. Whereas cellular reactivity (lymphocyte blastogenesis) for worm antigens was evident in mesenteric lymph nodes draining the gastrointestinal tract within three days after infection, Peyer's patch lymphocytes developed maximal reactivity two to three weeks later at the same time as the

spleen and other lymphoid tissues. Furthermore, the immune reactivity found in Peyer's patch lymphoid tissue did not appear to be the first site of cellular responsiveness but rather to acquire cellular reactivity only when other lymphoid elements in the infected host had also acquired similar antigen-induced reactivity.

Significance to Biomedical Research and the Program of the Institute:

These studies defining the molecular models of canine IgA's have made critical testing possible for the role of secretory piece in protecting secretory IgA from proteolysis and in a transport function. J-chain, likewise, is important in the assembly of IgA and probably IgM molecules. All these studies of canine IgA help to extend the homology of the mucosal secretory defense system in mammals and humans which is recognized as a major barrier for interaction with viral and bacterial micro-organisms by the host.

Studies on the function of gut lymphoid cells in birds and mammals, such as the Peyer's patch areas, are contradictory and may point to the tremendous species variations which will prevent easy generalities about function and significance. Therefore, each mammalian species will ultimately require study. The interaction of lymphoid cells which process antigens, such as gut bacterial antigens, and those which subsequently produce appropriate antibodies are likely to be vital in regulating the growth of normal intestinal flora and in the development of intestinal illnesses such as cholera and other diarrheal diseases. Now that these cells can be cultured in vitro and the various lymphocyte populations accurately identified, some of the confusion has been removed and hopefully more precise functions of these lymphoid aggregates can be found.

Proposed Course:

1. Further work on the structure of secretory piece and J-chain to show the interactions with IgA immunoglobulin and the elucidation of the chains' ultrastructure.

2. To elucidate the function of antigen processing cells in gut lymphoid tissue and to extend these observations to the bronchial associated lymphoid tissue.

Publications:

1. Levin, D. M., Wong, K., Reynolds, N. Y., Sutton, A., and Northrup, R. S.: Vi antigen from Salmonella typhosa and immunity against typhoid fever. II. Safety and antigenicity in man. Infect. Immun. 12: 1290-1294, 1975.

2. Thompson, R. E., Reynolds, H. Y., and Waxdal, M. J.: Structural composition of canine secretory component and immunoglobulin A. Biochemistry, 14: 2853-2860, 1975.

3. Levin, D. M., Ottesen, E. A., Reynolds, H. Y., and Kirkpatrick, C. H.: Cellular immunity in Peyer's patches of rats infected with Trichinella spiralis. Infect. Immun. 13: 27-30, 1976.

4. Strober, W., Krakauer, R., Kleaveman, H. L., Reynolds, H. Y., and Nelson, D. L.: Secretory IgA deficiency: a unique disorder of the IgA immune system. N. Eng. J. Med. 294: 351-356, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00045-08 LCI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Interaction of Antibody and Complement on the Production of Immune Damage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Frank	Head, Clinical Immunology Section	LCI	NIAID
OTHER:	J. A. Gelfand	Clinical Associate	"	"
	A. S. Fauci	Head, Clinical Physiology Section	"	"
	S. W. Hosea	Clinical Associate	"	"
	R. Reinhart	Biol. Lab. Tech. (BioChem.)	"	"
	T. Gaither	Research Biologist	"	"

COOPERATING UNITS (if any) I. Green and E. Shevach (LI, NIAID, NIH Z01 AI 00033-08 LI), M. Shin and R. Nagel (U Maryland, Baltimore, Md.), M. Gelfand (Dept. Nephrology, Georgetown U, Wash., D.C.), W. D. Terry (Immunology Branch, NCI, NIH), E. Jaffe (Pathology Branch, NCI, NIH)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-7/12	7/12	1

SUMMARY OF WORK (200 words or less - underline keywords)

Guinea pigs genetically deficient in the fourth component of complement show linkage between the gene controlling C4 synthesis and histocompatibility type. Demonstration of this provides the first animal model of linkage between a complement component in an animal and histocompatibility type. These animals now provide the only animal model for studies of complement synthesis in histocompatibility function. Other studies have shown immunoglobulins of the IgM class and gamma 1 and gamma 3 subclasses of IgG activate the alternative pathway. This was previously thought not to be the case. It was shown that at least half of the IgA myeloma proteins studied did not activate the alternative pathway. Also, this year, studies were continued using the IgG and complement sensitized red cell reagents to characterize and identify various kinds of lymphoid tumors in man. This whole approach has turned out to be of great importance and is leading to a thorough reclassification of human malignancies in collaboration with the NCI. We recently described a glomerular complement receptor. Patients who have immunoglobulin and complement deposition in the glomeruli in vitro, in a subepithelial location, have loss of their glomerulose complement receptor activity; and, therefore, it would appear that this receptor plays an important role in deposition of complement components in the glomeruli.

Project Description:

Objectives:

The objective of this program is the long-term study of the role of antibody in complement in the production of immune injury in vitro and in vivo. To this end, a variety of projects have been accomplished with a number of different investigators. These projects tend to fall into three major groups. The first group concerns the study of C4 synthesis and histocompatibility type. The second involves a number of problems related to complement receptors in tissue, their evaluation and function. This year, this set of problems has received most of our attention. The third set of problems involves complement activation and complement function in host defense mechanisms.

Methods Employed:

In all of these studies, the methods employed involved purification of complement components, their use in the sequential buildup of complement intermediates on cells, the identification of sera of patients with certain complement abnormalities so that the sera can be used as reagents, and the purification of immunoglobulins of various classes by standard techniques. Methods for the titration of complement components have been developed in our laboratory as well as in other laboratories, and these are in routine use.

Major Findings:

We have continued to extend our studies of the linkage of complement component synthesis and histocompatibility type and presented these findings to the complement workshop this year. In essence, these findings demonstrate that the gene controlling C4 synthesis is on the same chromosome as the genes which control the synthesis for histocompatibility antigens. The clear demonstration of this association means that we have developed in the C4 deficient guinea pig the only experimental model for studying this linkage between synthetic genes and histocompatibility type and a way of examining how this linkage pertains to the development of human disease. The fact of this linkage has been demonstrated in human beings and, therefore, is of great importance in terms of understanding complement function and in terms of understanding mechanism of disease.

The second project is concerned with receptor function on cells and tissues. We have continued our collaboration with the group in the pathology unit of the National Cancer Institute, and they have continued to reclassify the lymphoid malignancies on the basis of their IgG receptors, complement receptors, etc. This project is leading to a general reclassification of lymphoid malignancies and has assumed great importance in the general field of pathology. As part of this project, it has also been possible to identify nests of malignant cells in tissue section, and the techniques for identifying various cells with various receptors in tissue section have been refined in the past year. The refining of these techniques has involved the use of a

method that we developed last year. In this method, Salmonella typhi are directly fluorescinated and then allowed to react with fresh serum as a source of complement. Via the activation of the alternative complement pathway, these bacteria become coated with the complement component, C3b. Such complement coated bacteria are extremely useful reagents for identifying cells with C3b receptors in vitro and in frozen tissue section. Thus, for example, they can be used in suspensions of lymphoid cells to detect B lymphocytes with great ease. It is thought that this technique may generally replace the earlier EAC technique. We have also used these complement coated fluorescent bacteria on tissue section to demonstrate the so-called C3 complement receptor in glomeruli or the glomerular complement receptor that we described last year. The presence of this receptor may be very important in the development of human renal disease. One further step in the definition of the importance of this receptor was achieved in the past year. In collaboration with Dr. Michael Gelfand and the group of pathologists at the University of Maryland, we studied the activity of the glomerular complement receptor in a wide variety of human renal biopsy materials. It was shown that, in general, there was an inverse correlation between the activity of the glomerular complement receptor and the in vivo deposition of C3. Thus, patients with immunologic renal disease with complement deposited in their kidneys have a loss of the glomerular complement receptor. It was also possible to show, using the complement-coated bacteria and scanning electron microscopy, that this receptor is located on the renal epithelial cell and, in fact, is probably responsible for the subepithelial localization of complement in the kidney. It was also shown that patients who have complement deposition in areas other than subepithelial do not have complete loss of their glomerular complement receptors. We still do not know whether these receptors are responsible for immunologic damage, play a protective role, etc. However, these studies are underway. Another step in our understanding of immune complex disease was achieved this year by our further studies of the ability of various aggregated human myeloma proteins to activate the alternative or properdin complement pathway. Hithertofore, it had been believed that only IgA and perhaps IgE could activate this pathway when these proteins were added to sera, and this did not seem to make sense from the pathophysiologic point of view. In studies that have been in progress for some time, it was shown that, in fact, a variety of myeloma proteins can activate the alternative pathway. Most particularly, IgM complexes, IgG 1 and IgG 3 complexes were shown to activate this pathway. A few IgG 2 and 4 myelomas activated the alternative pathway. About half of the IgA myelomas studied activated the alternative pathway. The presence of a small amount of contaminating endotoxin within the preparations of myeloma proteins was shown to be very important in alternative pathway activation. Therefore, a series of myeloma proteins of the IgG 1 subclass (a subclass which was felt not to activate the alternative pathway) were prepared in an endotoxin-free manner. These endotoxin-free aggregated myeloma proteins activated this pathway. As a small side project in our studies of activation of the alternative pathway by bacterial products and immunoglobulin, a study was done with two patients with the Herxheimer reaction, a reaction following the injection of penicillin into patients with secondary syphilis. It was shown that the Herxheimer reaction is probably due to endotoxin release by the syphilis microorganism at the time that they

are killed by the penicillin. In collaboration with Dr. Elin, Dr. Jeffrey Gelfand demonstrated that these patients have free endotoxin in their serum at the time of this reaction, and that their complement components do not show major significant shifts in activity.

Also, this year, the project listed last year concerning the effect of complement on host defense against Candida organisms was completed. It was shown that complement plays a role in host defense against Candida infection. It was also shown that part of this role involved the clearance of the Candida organisms in the blood stream. Animals depleted of complement activity have a defect in their ability to clear Candida organisms. However, it was shown that this defect is relatively minor. Of much greater significance was the finding that once the Candida organisms are in the tissues, the decomplemented animals do not have adequate ability to fight the Candida infection. The organism overgrows and kills the animals.

Proposed Course:

The various aspects of these projects will be continued with emphasis on the pathophysiology of complement action and the continued study of the role of receptors for complement fragments in the pathogenesis of disease and in host defense mechanisms.

Publications:

1. Frank, M. M., May, J. E., and Kane, M. A.: Studies of the biological effects of selective C4 deficiency. In Good, R. (Ed.): Proceedings of the Second International Conference on Immunodeficiency Diseases in Man. Birth Defects: Original Article Series 11: 567-570, 1975.

2. Green, I., Jaffe, E. S., Shevach, E. M. Edelson, R. L., Frank, M. M., and Berard, C. W.: Determination of the origin of malignant reticular cells by the use of surface membrane markers. Int. Acad. of Pathol. Monograph. The Reticuloendothelial System. Rebuck, J. W., Berard, C. W. and Abell, M. R. (Eds.) Baltimore, Md., Williams & Wilkins Co., 1975, pp 282-301.

3. Jaffe, E. S., Shevach, E. M., Sussman, B. S., Frank, M. M., Green, I., and Berard, C. W.: Membrane receptor sites for the identification of lymphoreticular cells in benign and malignant conditions. Brit. J. Cancer 31, Suppl II, pp 107-120, 1975.

4. Rothman, I. K., Gelfand, J. A., Fauci, A. and Frank, M. M.: The Immune Adherence Receptor: Dissociation between the expression of erythrocyte and mononuclear cell C3b receptors. J. Immunol. 115: 1312-1315, 1975.

5. Gelfand, M. C., Frank, M. M., and Green, I.: A receptor for the third component of complement in the human renal glomerulus. J. Exp. Med. 142: 1029-1035, 1975.

6. Frank, M. M.; Current concepts in complement research. Scope Monograph, 1975.

7. Gelfand, J. A., Fauci, A. S., Green, I. and Frank, M. M.: A simple method for the determination of complement receptor-bearing mononuclear cells. J. Immunol. 116: 595-599, 1976.

8. Frank, M. M., Jaffe, E. S. and Green, I.: Detection of specific mononuclear cell receptors in tissue sections. In David, J. and Bloom, B. (Eds.): In Vitro Methods of Cell Mediated Immunity. New York City, Academic Press. In press.

9. Gelfand, M. C., Shin, M. L., Nagle, R. B., Green, I. and Frank, M. M.: The glomerular complement receptor in immunologically mediated renal glomerular injury. N. Engl. J. Med. In press.

10. Gelfand, J. A., Elin, R. J., Berry, F. W. and Frank, M. M.: Endotoxemia associated with the Jarisch-Herxheimer reaction of syphilis. N. Engl. J. Med. In press.

11. Braylan, R. C., Jaffe, E. S., Burbach, J. W., Frank, M. M., Johnson, R. E. and Berard, C. W.: Similarities of surface characteristics of neoplastic well-differentiated lymphocytes from solid tissues and from peripheral blood. Cancer Res. In press.

12. Jaffe, E. S., Braylan, R. C., Frank, M. M., Green, I., Berard, C. W.: Heterogeneity of immunologic markers and surface morphology in childhood lymphoblastic lymphoma. B. J. Cancer. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00046-08 LCI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pathogenesis of Delayed Hypersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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S. Chapman, Clinical Associate, LCI, NIAID
P. Hoffman, Guest Worker, LCI, NIAID
M. Frank, Head, Clinical Immunology, LCI, NIAID
J. Gallin, Senior Investigator, LCI, NIAID
D. Wright, Clinical Associate, LCI, NIAID
H. Reynolds, Head, Bacterial Diseases Section, LCI, NIAID
T. Smith, Biol. Lab. Tech. (Micro.), LCI, NIAID
L. Robinson, Co-Step, LCI, NIAID

COOPERATING UNITS (if any)

R. Edelson (LI, NIAID, NIH)
J. Cook and A. Lewis (LVD, NIAID, NIH) Z01 AI 00013-14 LVD
D. Poplack (POB, NCI, NIH)

LABORATORY

Laboratory of Clinical Investigation

SECTION

Clinical Allergy and Hypersensitivity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5- 7/12	4- 4/12	1- 3/12

SUMMARY OF WORK (200 words or less - underline keywords)

These studies have been directed at answering three general questions: 1) definition of the contribution of cell-mediated immune mechanisms to resistance of humans to certain opportunistic infections; 2) evaluation of immunologic reconstitution as an therapeutic tool in management of patients with certain opportunistic infections; and 3) identification and characterization of the components of dialyzable transfer factor.

The results obtained show that approximately 66% of patients with chronic mucocutaneous candidiasis have definable defects in cellular immunity. In most cases, these defects can be corrected by treatment with transfer factor. Correction of the defect alone is not associated with clinical benefits. However, if the infections are cleared with antibiotics and transfer factor is used as a form of consolidation therapy, sustained remissions are observed.

Transfer factor contains many components some of which mediate antigen-dependent effects while others appear to serve as modulators of the antigen-dependent reactions.

PHS-6040
(12-75)

Project Description:

Objectives (sub-project A):

1) Characterization of cellular immune responses in immunologically deficient patients with chronic infectious diseases;

2) Studies of the pathogenesis of acquired and congenital immunologic deficiencies;

3) Characterization of the pathogenesis of cutaneous inflammation in chronic mucocutaneous candidiasis;

4) Establishment of an animal model for cutaneous candidiasis for study of mechanisms of inflammation in the skin;

5) Evaluation of levamisole on cellular immune and chemotactic responses by immunologically deficient patients.

Methods Employed:

The major portion of our studies of cellular immune deficiency have been conducted in patients with chronic mucocutaneous candidiasis. Approximately 35 patients with this syndrome have been evaluated by our group; evidence of immunologic deficiency has been found in 66% of the patients. The general model for the pathogenesis of cellular immunodeficiency that we presented in 1971 still serves as a valid outline in which to conduct studies of host-defenses. According to this model patients with candidiasis developing during infancy or early childhood have a congenital basis for their immunologic deficits.

Recently we have seen patients who did not have evidence of immunodeficiency during childhood or early adult life. Instead, they developed classical mucocutaneous candidiasis during the fifth and sixth decade. These patients have been used to study mechanisms of acquired cellular immunodeficiency, using a modification of our previously reported methods for evaluating lymphocyte functions in vitro.

In our 1971 model we proposed that lymphocytes may respond to antigens by replicating (lymphocyte transformation), producing lymphokines or developing cytotoxic activity. The first two responses have received intensive evaluation in our laboratory and data have been generated, both by ourselves and by others, that suggest that these responses may involve separate subpopulations of T-cells. Now, in collaboration with Dr. David Poplack, POB, NCI, we have undertaken a study of cytotoxic activities of antigen and mitogen-stimulated lymphocytes from patients with chronic mucocutaneous candidiasis.

We have also investigated the mechanisms of inflammation in the skin of patients with chronic candidiasis. This study was undertaken in an effort to define the inflammatory pathways that were operative in patients with deficient cellular immunity, but who had intense cutaneous inflammation. Preliminary experiments suggested that the complement system was being activated directly via the alternative pathway.

Prompted by these findings we have established two models for acute cutaneous candidiasis in guinea pigs. One involves placement of viable yeast-phase organisms under occlusive dressings. The second does not employ occlusion but larger numbers of organisms are rubbed onto the shaved skin. The pathogenesis of these infections have been followed with conventional as well as fluorescence microscopy. The immune status of the animals has also been assessed with both in vivo and in vitro techniques.

The studies of immunologic components of host defenses have been extended to a tumor system in collaboration with Drs. James Cook and Andrew Lewis, LVD, NIAID. These experiments have been conducted in hamsters and initial efforts have involved development of assays for T-cell immunity in hamsters. These studies are essentially complete and we are now examining the natural history of tumors in normal and thymectomized mice.

Levamisole is an antihelminthic drug that through serendipity was found to amplify immune responses. In collaboration with Drs. Daniel Wright and John Gallin we have examined the effect of levamisole on chemotactic responses by neutrophils and monocytes from normal volunteers and patients with chemotactic disorders.

Major Findings:

In the annual report for 1974-1975, we summarized the evidence for a congenital basis for the immune deficiencies in patients with the early onset form of chronic mucocutaneous candidiasis. We also stated that the mechanism of the underlying immunologic abnormality in patients with late onset candidiasis was unknown. Now, there is evidence that patients with late onset candidiasis have an active suppressive factor that is related to the immune defect and perhaps to the predisposition to the disease.

A 56 year old woman with a three-year history of candidiasis had cutaneous anergy and her lymphocytes failed to respond to antigens in vitro. When her lymphocytes were cultured without antigen for several days, washed and then stimulated with antigen, normal responses were observed. Addition of freshly collected cells to the responsive cells suppressed these responses.

Qualitatively similar results have been obtained with another late onset candidiasis patient.

The mechanism of this effect is cellular but is otherwise unclear. It is still under study.

Previously reported work has shown that most candidiasis patients have normal numbers of T-cells and T-cell responses to plant mitogens are normal; only responses to antigens are deficient. Although the work is still preliminary, the data obtained so far indicate that mitogen-induced cytotoxicity is normal. Antigen-induced T-cell cytotoxicity is currently under study.

The study of mechanisms of inflammation in candidiasis patients have been rewarding. These studies were conducted by Dr. Peter Sohnle and have defined two mechanisms for clearing candida organisms from the skin of guinea pigs. In infections established under occlusive dressings there is prompt infiltration with neutrophils. A crust of epidermis, organisms and neutrophils forms and is sloughed, following which the infected area heals. The immunofluorescent studies have shown complement (C3) along the basement membrane zones. Immunoglobulins and C4 have not been found. In vitro studies have shown that a component of the organism (probably the mannan) can activate complement via the alternative pathway and thereby generate chemotactic factors. It has also been shown that the immune status of the animal does not affect the clinical course of the reactions in these infections.

The second model for acute candidiasis does not require occlusion. In these animals one sees small abscesses and slow formation of a hyperkeratotic crust. The cellular infiltrate is predominantly mononuclear and immunofluorescent studies do not show complement. By the time these infections have produced marked scaling, the animals have developed positive delayed skin tests to candida. The role of delayed hypersensitivity in the clinical course of these infections has been studied. In immune animals, the scaling process and clearance of the organisms is markedly accelerated. Moreover, accelerated reactions are seen in animals that have been passively sensitized with lymphoid cells from immune donors. Immune serum does not course accelerated clearance of the organisms or rapid formation of scaling.

The studies of tumor immunity in hamsters are just beginning. A system for studying lymphocyte responses in vitro has been developed. The effects of thymectomy and thymectomy followed by thymus grafting on these responses is underway.

The studies with levamisole have shown concentrations in the range of attainable blood levels increase in vitro neutrophil and mononuclear cell responses to chemotactic stimuli. The same concentrations of drug increase cell metabolism and mononuclear cell cyclic quanosine monophosphate. It is not clear that these events are interrelated.

Four patients with chemotactic defects were given levamisole; each showed improved cellular chemotaxis. Administration of the drug was not continued and studies on resistance to infections have not yet been done.

Significance:

1) These results and the data described in sub-project B show the important relationship of cell mediated immunity to susceptibility to certain opportunistic pathogens.

2) The findings of a cellular suppressor activity in patients with late-onset candidiasis may be an important clue to the mechanism of the immune deficiency in patients with this form of candidiasis.

3) The findings in the skin biopsies from the patients indicate that antibodies or immune complexes are probably not involved in the inflammatory responses in mucocutaneous candidiasis. Thus, the patients are susceptible to infection because of the defect in cellular immunity, but develop lesions because of normal functions of the complement system. These data are supported by the findings with acute infections in guinea pigs.

4) The finding that the rate of scaling and clearance of organisms from the skin of animals infected without occlusive dressings is related to delayed hypersensitivity provides additional evidence for involvement of the cellular immune system in resistance to this organism. Moreover, the relationship of a scaling dermatosis to delayed hypersensitivity is important to a general problem in dermatology. Many scaling reactions have been pressured to be due to delayed hypersensitivity. Our observations show that, in certain cases, this supposition is correct.

5) Although many patients with recurrent bacterial infections have been found to have impaired cellular chemotaxis, the relationship between the clinical observation and the laboratory finding is still unclear. Levamisole may provide us with a method for correction of chemotactic defects and observing the patients for improved resistance to infection.

Proposed Course:

Each of these studies will be continued. Specifically, Dr. Sohnle plans to extend his model to oral infections with candida. A clinical study of levamisole in patients with chemotactic defects is planned.

Objectives (sub-project B):

1) Evaluation of immunologic reconstitution as a therapeutic adjunct for treating chronic infectious diseases;

2) Identification and characterization of the components of dialyzable transfer factor and assessment of their functions in immunologic and inflammatory reactions;

3) Investigation of transfer factor-like materials in laboratory animals.

Methods Employed:

The trial of transfer factor in candidiasis is being continued with the oldest participants being in the fifth year of treatment. These subjects receive transfer factor every four months.

A new study has just been started in which immunodeficient patients with candidiasis are being treated with transfer factor from candida skin test negative donors.

Whole dialyzable transfer factor is being separated into its component parts by gel filtration, ion-exchange chromatography and silica gel chromatography. Fractions are assayed for composition and activities on immune responses in vivo and in vitro.

Dr. Peter Hoffman has undertaken development of a transfer factor system in guinea pigs. Preliminary experiments indicate that his cell lysates are able to transfer contact allergic reactions to insensitive recipients.

Major Findings:

Currently six candidiasis patients are receiving transfer factor from donors with positive skin tests. All are still in remission for durations of 5, 5, 4, 2, 1 and 1/2 years respectively.

Three subjects are receiving transfer factor from candidia-negative donors, but it is too soon to make an evaluation.

By gel filtration we have obtained a fraction of crude transfer factor that contains the property of transferring delayed skin reactivity to candidiasis patients. This fraction was unique in that it strongly absorbed 255 nm ultra-violet light. This property has been considered by some workers as indicative of nucleic acid material. We have recently found that this absorbance is due to hypoxanthine. Xanthine oxidase-treated transfer factor still transfers skin reactivity to rhesus monkeys so transfer factor is not hypoxanthine. The digested transfer factor contains some peptides and we are currently working on separation and identification of them.

Dr. John Gallin and I have previously reported that transfer factor contains chemotactic activity. We are now fractionating the chemotactic activity in an attempt to identify the chemotactic substances. At this time the evidence suggests that they are small polypeptides.

Significance:

These studies represent progress toward identification of the active component(s) of transfer factor. Our model proposes that transfer factor contains several properties, some of which are responsible for the antigen-dependent (and apparent antigen-specific) activities and others are responsible for the antigen-independent properties such as chemotaxis and cGMP

accumulation that modulate the antigen-dependent activities. Identification of the components will be an important step toward evaluation of the hypothetical model and determination of the mechanism of action of transfer factor.

Proposed Course:

These studies will be continued. Specifically, we are planning to study the effects of enzyme degradation on activities of transfer factor.

Publications:

1. Axelsen, N. H., Kirkpatrick, C. H., and Buckley, R. H.: Precipitins to Candida albicans in chronic mucocutaneous candidiasis studied by crossed immunoelectrophoresis with intermediate gel. Correlation with clinical and immunological findings. Clin. Exp. Immunol. 17: 385-394, 1974.

2. Edelson, R. L., Lutzner, M. A., Kirkpatrick, C. H., Shevach, E. M., and Green, I.: Morphologic and functional properties of the atypical T lymphocytes of the Sezary syndrome. Mayo Clin. Proc. 49: 558-566, 1974.

3. Edelson, R. L., Kirkpatrick, C. H., Shevach, E. M., Schein, P. S., Smith, R. W., Green, I., and Lutzner, M.: Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes. Morphologic and functional studies. Ann. Intern. Med. 80: 685-692, 1974.

4. Graw, R. G., Jr., Lohrmann, H. P., Bull, M. I., Decter, J., Herzig, G. P., Bull, J. M., Leventhal, B. G., Yankee, R. A., Herzig, R. H., Krueger, G. R. F., Bleyer, W. A., Buja, M. L., McGinniss, M. H., Alter, H. J., Whang-Peng, J., Gralnick, H. R., Kirkpatrick, C. H., and Henderson, E. S.: Bone-marrow transplantation following combination chemotherapy immunosuppression (B.A.C.T.) in patients with acute leukemia. Transplant. Proc. VI 349-354, 1974.

5. Pachman, L. M., Krikpatrick, C. H., Kaufman, D. B., and Rothberg, R. M.: The lack of effect of transfer factor in thymic dysplasia with immunoglobulin synthesis. J. Pediatr. 84: 681-688, 1974.

6. Kirkpatrick, C. H.: Properties and activities of transfer factor. J. Allergy Clin. Immunol. 55: 411-421, 1975.

7. Kirkpatrick, C. H.: Restoration of cell-mediated immune responses with transfer factor. Birth Defects: Original Article Series XI: 441-444, 1975.

8. Kirkpatrick, C. H., Wells, S. A., Burdick, J. F., and Smith, T. K.: Effects of fetal thymus transplantation on defective cellular immunity. Birth Defects: Original Article Series XI: 367-369, 1975.

9. Kirkpatrick, C. H. and Rosenthal, A. S.: Corticosteroids and immunologic reactions. In Azarnoff, D. L. (Ed.): Steroid Therapy. Philadelphia, W. B. Saunders Publications, 1975, pp. 238-255.

10. Lutzner, M., Edelson, R., Schein, P., Green, I., Kirkpatrick, C. H. and Ahmed, A.: Cutaneous T-cell lymphomas: The Sezary syndrome, mycosis fungoides and related disorders. Ann. Int. Med. 83: 534-552, 1975.

11. Sandler, J. A., Smith, T. K., Manganiello, V. C. and Kirkpatrick, C. H.: Stimulation of monocyte cGMP by leukocyte dialysates: An antigen-independent property of dialyzable transfer factor. J. Clin. Invest. 56: 1271-1279, 1975.

12. Kirkpatrick, C. H.: Asthma and atopic hypersensitivity. In Kirkpatrick, C. H. and Reynolds, H. Y. (Eds.): Immunologic and Infectious Reactions in the Lung. New York, M. Dekker, 1976, pp. 211-228.

13. Kirkpatrick, C. H.: Cancer and immunodeficiency diseases. In Bergsma, D. (Ed.): Cancer and Genetics. New York, Alan R. Liss, Inc. 1976, pp. 61-78.

14. Kirkpatrick, C. H.: Mitogen and antigen-induced lymphocyte responses in patients with infectious diseases, In Role of Mitogens in Immunology. New York, Academic Press. 1976, pp. 639-655.

15. Kirkpatrick, C. H., Ottesen, E. A., Smith, T. K., Wells, S. A. and Burdick, J. F.: Reconstitution of defective cellular immunity with fetal thymus and dialyzable transfer factor: long term studies in a patient with chronic mucocutaneous candidiasis. Clin. Exp. Immunol. 23: 414-431, 1976.

16. Kirkpatrick, C. H., and Reynolds, H. Y. (Eds.): Immunologic and Infectious Reactions in the Lung. New York, M. Dekker, Inc., 1976, pp. 1-589.

17. Sohnle, P. G., Frank, M. M. and Kirkpatrick, C. H.: Deposition of complement components in the cutaneous lesions of chronic mucocutaneous candidiasis. Clin. Immunol. Immunopath. 5: 340-350, 1976.

18. Ascher, M. S., Gottlieb, A. A., and Kirkpatrick, C. H. (Eds.): Transfer Factor. Basic Properties and Clinical Applications. New York, Academic Press. 1976. In press.

19. Kirkpatrick, C. H. and Smith, T. K.: Effects of dialyzable transfer factor on accumulation of cyclic nucleotides by leukocytes. In Ascher, M. S., Gottlieb, A. A. and Kirkpatrick, C. H. (Eds.): Transfer Factor. Academic Press. In press.

20. Kirkpatrick, C. H., Robinson, L. G. and Smith, T. K.: The identification and significance of hypoxanthine in dialyzable transfer factor. Cell. Immunol. In press.

21. Kirkpatrick, C. H., and Smith, T. K.: The nature of transfer factor and its clinical efficacy in the management of cutaneous disorders. J. Invest. Derm. In press.

22. Kirkpatrick, C. H.: Transfer factor for mucocutaneous candidiasis, In Recent Advances in Dermatopharmacology. In press.

23. Sohnle, P. G., Frank, M. M. and Kirkpatrick, C. H.: Mechanisms involved in elimination of organisms from experimental cutaneous Candida albicans infections in guinea pigs. J. Immunol. In press.

24. Steinberg, A. D. and Kirkpatrick, C. H.: Concepts of immunologic intervention in the treatment of asthma. In Siegal, M. S. and Weiss, E. B. (Eds.): Bronchial Asthma, Mechanisms and Therapuetics, Little, Brown and Co. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00047-07 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Clinical Studies on Patients with Known or Suspected Parasitic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	E. A. Ottesen	Senior Investigator, Clin. Parasitol. Section (dual position)	LCI	NIAID
	D. D. Metcalfe	Clinical Associate	LCI	NIAID
	J. A. Gelfand	Clinical Associate	LCI	NIAID

COOPERATING UNITS (if any) B. Amos and F. Ward (Duke Univ. Medical Center, Durham, N.C.); R. Hiatt (San Juan Lab., Center for Dis. Control, Atlanta, Ga.); Z. R. Sotomayor (Univ. Puerto Rico School of Med., San Juan, P.R.); Medical Office (Peace Corps, Wash., D. C.); P. F. Weller (LPD, NIAID, NIH); (Con't. below)

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-5/12	2-5/12	--

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been completed of a population on the island of Mauke (Cook group, South Pacific) where W. bancrofti filariasis is endemic. Individuals without microfilaremia exhibited strong cellular immune responses to filarial antigens by lymphocyte transformation while those with microfilaremia were poorly responsive. This defect was specific for filarial antigens and did not involve humoral antibody responses.

A similar pattern was found in patients with schistosomiasis. Cases studied within three months of initial infection with S. mansoni generated brisk in vitro cellular immune responses to schistosome antigens, whereas lymphocytes from those with chronic infection had a diminished response to the same antigens.

Among patients seen in consultation, a case of babesiosis was diagnosed and the organism isolated from an infection acquired on Martha's Vineyard, an island off Massachusetts.

COOPERATING UNITS (Con't.):
L. H. Miller (LPD, NIAID, NIH) (Z01 AI 00105-04); A. W. Cheever, (LPD, NIAID, NIH) (Z01 AI 00092-10); F. A. Gill (Bethesda, Md.)

PHS-6040
(12-75)

Project Description:

Studies were completed on the data and specimens brought back from the filariasis field project conducted on Mauke island of the Cook group in the South Pacific. A new investigation was initiated in schistosomiasis patients with focus upon cellular immune responses at different stages of their infection. The consultation and outpatient services for parasitic diseases continue to grow and provide a potential source of clinical material for investigation as well as teaching purposes for the LCI staff.

The finding of greatest interest in the filariasis study was the difference in cellular immunity as measured by lymphocyte transformation between a population of non-filaremic individuals as compared to those with patent microfilaremia. Since there is abundant exposure to filariasis among the entire population of this isolated small island, we interpret the results as indicating that initial exposure to parasites elicits a vigorous cellular immune response but continued exposure or reinfection desensitizes the host to a non-responsive state. This reduced cellular responsiveness in the microfilaremic cases was shown to be specific for filarial antigens and not associated with impaired humoral antibody response. A number of associated issues were also examined in the filariasis field study including clinical and immunologic testing before and after diethylcarbamazine treatment of patients, tissue typing (HLA) of filariasis patients, eosinophil responses associated with treatment, and assessment of skin tests with dirofilarial antigen (Ottesen and Weller).

In collaboration with groups in Puerto Rico, studies of cellular immune reactivity were initiated in schistosomiasis patients. Cases at different stages of their infection, as early as within three months, have been studied. A pattern somewhat similar to that in filariasis was found in that brisk in vitro lymphocyte reactivity to schistosome antigens was found in recently infected patients while those with chronic infections had greatly diminished responsiveness. No obvious immunologic abnormality was found that might help explain the striking and severe systemic symptoms experienced by the cases of acute schistosomiasis. One rather surprising finding in the cases with early or acute schistosomiasis was the poor correlation between severity of illness and intensity of infection as judged by quantitative fecal egg counts (Ottesen, Cheever, and Neva).

The following eosinophile functions are being studied in collaboration with others: ability of parasite antigen and antibody to stimulate release of certain eosinophil enzymes (Metcalfe and Ottesen); the presence of receptors for immunoglobulin and complement components on eosinophils (Ottesen and Gelfand).

Laboratory confirmed diagnosis of the first case of human babesiosis from Martha's Vineyard, Massachusetts, was made in a patient seen in consultation at a local hospital for suspected malaria. Half a dozen other cases had occurred a few months earlier on the neighboring island of Nantucket. These cases of a tick-transmitted protozoan parasite that invades red blood cells and produces a febrile hemolytic anemia are of particular interest because they represent a small but definite outbreak of a hitherto rare human infection. All

Massachusetts cases occurred in individuals with presumably intact spleens and are believed to be Babesia microti; previous cases have occurred in splenectomized people and involved other species (Neva and Miller).

The studies directly related to the filariasis field project in the Pacific are essentially completed but some experimental work on filariasis in animal models will be continued. The possibilities for collaborative work in India on tropical eosinophilia are currently being explored. It is hoped that additional cases of acute schistosomiasis may become available so they can be studied while in the symptomatic period; further observations will be made upon chronic cases when encountered. Experimental chemotherapy with the babesial parasite and field studies on babesial strains will be carried out by others.

Publications:

1. Neva, F. A., Kaplan, A. P., Pacheco, G., and Gray, L.: Tropical eosinophilia. A human model of parasitic immunopathology, with observations on serum IgE levels before and after treatment. J. Allergy Clin. Immunol. 55: 422-429, 1975.

2. Raizman, R. E., Neva, F. A., Eckert, R. J., Duffy, P. A., and Lipshutz, W. H.: Chagasic mega-esophagus: similarity to achalusia by manometrics, radiography and response to pneumatic dilatation. Am. J. Digest. Dis. 20: 882-888, 1975.

3. Ottesen, E. A., Smith, T. K., and Kirkpatrick, C. H.: Immune response to Trichinella spiralis in the rat I. Development of cellular and humoral responses during chronic infection. Int. Arch. Allergy 49: 396-410, 1975.

4. Ottesen, E. A.: Eosinophilia and the lung. In Kirkpatrick, C. H. and Reynolds, H. Y. (Vol. Eds.): Immunologic and Infectious Reactions in the Lung, Vol. 1, Lung Biology in Health and Disease. Claude Lenfant, Exec. Ed. New York, Marcel Dekker, Inc., 1976, Chap. 15, pp. 289-332.

5. Levin, D. M., Ottesen, E. A., Reynolds, H. Y., and Kirkpatrick, C. H.: Cellular immunity in Peyer's patches of rats infected with Trichinella spiralis. Infect. Immun. 13: 27-30, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00048-06 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Pathophysiology of Autoimmune Hemolytic Anemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	C. Jaffe	Clinical Associate	"	"
	H. Reynolds	Head, Bacterial Diseases Section	"	"

COOPERATING UNITS (if any) M. McGinniss (Blood Bank, CC), J. Vierling and A. Jones (DDB, NIAMDD; Project No.: Z01 AM 53503-02 DDB), J. Atkinson (Dept. Med., Barnes Hospital, St. Louis, Mo.)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1-4/12	8/12

SUMMARY OF WORK (200 words or less - underline keywords)

Clearance of IgM sensitized RBC is entirely dependent on cell bound C3b. C3d coated cells do not get cleared from the circulation if IgM is the antibody responsible for their attachment. There are a limited number of sites for C3 binding when the C3 is activated by cold agglutinin antibodies, and these sites differ for cold agglutinins and isoagglutinins. Cells with these sites occupied by C3d, the nonhemolytic fragment of C3, are less susceptible to lysis than normal cells. Rh antibodies were shown to require the classical complement pathway for optimal clearance; the fragment responsible for clearance of the cells was shown to be C3d. Thus, C3d cooperates with IgG antibody but not with IgM to produce clearance. Initial studies using the techniques developed in this program in examining other diseases have been completed and immunospecific defect in the clearance of particles from the circulation has been shown in patients with primary biliary cirrhosis.

Project Description:

Objectives:

To define the pathophysiologic function of antibody and complement on the surface of red cells in patients with acute hemolytic anemia and to determine how the body handles particles coated with antibody and complement components when these particles are in the circulation. To determine whether this handling of particles is normal in patients with diseases other than those of autoimmune hemolytic anemia as a way of understanding the pathophysiologic disorder in these other conditions.

Methods Employed:

These studies examine the clearance of radiochromated erythrocytes from the circulation. The initial studies were performed in a guinea pig model but almost all of the recent studies have been performed in human volunteers or in patients with various diseases. In the studies that have been the focus in the past year, erythrocytes coated with IgM isoagglutinin antibodies or IgM cold agglutinin antibodies were examined. In the case of isoagglutinin, the sensitized cells are injected into the circulation, and their clearance followed. In the case of cold agglutinin sensitized cells, the cells coated with antibody are allowed to react with serum complement in vitro and then the antibody is washed off the cells at 37° and the complement coated cells are injected into the circulation in the absence of antibody. In other experiments, radioactive cells are coated with Rh antibody of the IgG class which has been highly purified, and the survival of these cells is examined. The third general set of clearance studies examines the clearance of radioactive albumin which has been aggregated. This is the standard method for determining RES clearance and can be used to determine both liver blood flow and the activity of the reticuloendothelial system. During this past year, we have completed our studies of cold agglutinin sensitized cells and their clearance from the circulation. A whole series of findings have been submitted for publication or presented at various scientific meetings. These findings include the following: The fragment responsible for the clearance of cells coated with cold agglutinin and complement from the circulation would appear to be the fragment C3b. The cleavage fragment, C3d, does not mediate the clearance of cold agglutinin sensitized cells. The complement fragment leads to the sequestration of the cold agglutinin sensitized cells in the liver. Following sequestration in the liver, a portion of the cells are phagocytosed and a portion of the cells are released back into the circulation where they survive normally with the cleavage fragment, C3d, on their surface. C3d does not mediate the clearance of cold agglutinin sensitized cells. It was also shown that activation of the complement leads to C3b deposition, and this in turn reacting with the C3b inactivator leads to the formation of cells coated with C3d. Cells coated with C3d are actually protected from destruction within the circulation.

The studies also showed that cold agglutinin on cells leads to deposition of C3d in very specific sites on the erythrocyte surface. When

such sites are occupied, they preclude the further deposition of C3b. Nevertheless, isoagglutinins can place C3b on the erythrocytes at other sites. Thus, C3b does not travel far in terms of its attachment to erythrocyte surfaces. During the past year, studies have proceeded with anti-Rh D and its effect on red cell survival. The clearance of cells sensitized with IgG anti-Rh D has been studied. Hitherto, it was generally accepted that the clearance of these cells was independent of complement action. This year, we demonstrated that complement is indeed required for the clearance of these cells. However, the complement fragment which mediates cell clearance is an unusual fragment which hitherto in our studies has not been shown to effect the clearance of erythrocytes (C3d). Moreover, this fragment is not identified by standard, commerical Coombs' sera. Thus, previous studies of cells sensitized with Rh antibodies have suggested that complement was not involved in the clearance of these cells. In our studies, we have identified small amounts of C3d on the erythrocytes. We have shown that this C3d augments phagocytosis of the erythrocytes by phagocytic cells in the test tube, and more to the point have shown in six individuals with low levels of C4 (i.e., less than one-tenth of one percent of the normal serum level) that the clearance of these cells was markedly abnormal. Interestingly, in the many dozens of patients and normal volunteers that we have done, the clearance was only abnormal in these six individuals. All of the other patients had normal levels of the early complement components. Thus, we have a striking new finding in immunohematology but, more important, have been able to define a function for the complement fragment, C3d. It will be of great importance now to find what the general function is of C3d acting with immunoglobulin G in host defense reactions. As part of these studies of the pathophysiology of autoimmune hemolytic anemia, we have developed normal values for the clearance of C3b coated erythrocytes from the circulation, for the clearance of IgG and C3d coated erythrocytes from the circulation, and finally have defined normal values for the clearance of aggregated albumin, the standard method of testing the RES clearance. It has become clear that these tests are not only of interest in our studies of the pathophysiology of autoimmune hemolytic anemia. For example, many diseases appear to be due to circulating immune complexes. In some diseases such as systemic lupus erythematosus, these circulating complexes which ordinarily would be cleared by the liver are deposited in renal tissue, and this deposition of renal damage is what is responsible for the high mortality in this disease. It has been postulated by many workers that the RES of the liver is overloaded in patients with this and other diseases; but, in fact, such overload has never been demonstrated by the standard tests of RES function. It has occurred to us that the techniques we have developed can be used to more generally define the function of the reticuloendothelial system and its status in man. Thus, we have studied the clearance of C3b coated erythrocytes, Rh IgG coated erythrocytes and aggregated albumin in three groups of patients, two of whom have putative immunologically mediated diseases. The three groups of patients chosen for study were patients with primary biliary cirrhosis, chronic active hepatitis, and, as a control group, alcoholic cirrhosis. There are those who believe that the first two groups might have an immunologic basis. Our initial studies have been completed and show that patients with primary biliary cirrhosis, but not the other two groups of patients, have a specific defect

in their ability to clear C3b coated particles from their circulation. Their clearance of IgG coated particles is, if anything, increased. This inability to clear C3b coated particles is not a function of the inability to put complement on cells since the levels of their complement components are if anything elevated. Thus, it would appear that we have been able to develop new techniques for studying the reticuloendothelial system. It is clear from our studies already that these techniques test other functions of the RES than does the clearance of aggregated albumin. For example, the clearance of aggregated albumin was normal in each of these patients with primary biliary cirrhosis who had an abnormality in the clearance of IgG coated particles and C3b coated particles. We plan to extend these findings to other diseases and attempt to explore how immunologic clearance mechanisms can be defined and help us to explain the basis for some of these diseases. We believe that we are testing receptor function (i.e., the receptors on the surface of cells of the reticuloendothelial system) and that these tests of receptor function may turn out to be very valuable adjuncts for the study of diseases in man. These studies will be extended to other diseases in man in the next years, particularly those with autoimmune or immunologic basis.

Publications:

1. Atkinson, J. P. and Frank, M. M.: The use of glucocorticoids in the treatment of hemolytic disorders. In Azaroff, D. (Ed.): Steroid Therapy. Philadelphia, Pa., Saunders and Co., 1975, pp 49-61.

2. Frank, M. M., Schreiber, A. D., Atkinson, J. P. and Jaffe, C. J.: Pathophysiology of autoimmune hemolytic anemia. Ann. Int. Med. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00049-06 LCI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunobiologic and Ultrastructural Characterization of the Mechanisms of Antigen Recognition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. T. Blake	Bio. Lab. Tech. (Micro.)	LCI NIAID
	K. U. Cehrs	Bio. Lab. Tech. (Micro.)	LCI NIAID

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

SUMMARY OF WORK (200 words or less - underline keywords)

The interests and proposed course of the Biologic Structure Section is to characterize the role of the macrophage in antigen recognition by T lymphocytes in the expression and initiation of cell mediated immunity in man and experimental animals. In addition, we are interested in exploring the role of histocompatibility and genetic regulation in cell-cell interactions required for expression of cell mediated immunity. The Biologic Structure Section is also actively characterizing the physical state of, localization, and fate of macrophage associated antigen in in vitro antigen mediated lymphocyte proliferation and determining if genetic control of the immune response to insulin exists in man.

Project Description:

Objectives:

1) To characterize in a quantitative and qualitative fashion the role of macrophages and lymphocytes in a variety of in vitro correlates of cellular immunity such as antigen-dependent lymphocyte proliferation and macrophage inhibitory factor production.

2) To study in man and experimental animals the mechanism of lymphocyte antigen recognition using defined antigens such as insulin.

3) Characterize the role of macrophage-lymphocyte interaction in regulation of antigen recognition by thymus-derived and bone-marrow derived lymphocytes.

4) To study the cellular and molecular basis of the genetic control of immune responsiveness and the role of surface membrane determinants in cell cooperation.

Methods Employed:

1) Determination of DNA synthesis of lymphocytic populations with and without addition of antigen by 4 hour pulse of ^{3}H-TdR incorporation. Results assayed by either/or both autoradiography or liquid scintillation spectrometry.

2) Preparation and isolation of partially purified population of lymphocytes from oil-induced peritoneal exudates or regional draining nodes subsequent (2-4 weeks) to immunization of inbred strain 2 and 13 guinea pigs with chemically defined antigens such as horse spleen ferritin, horseradish peroxidase or hapten protein conjugates.

3) Column purification: Peritoneal exudate cells (PEC) or lymph node cell suspensions were passed over glass bead columns and incubated at 37°C for one hour. Cells were then eluted with 10% guinea pig sera in minimal essential Eagle's media and the recovered cells studied.

4) Preparation of macrophage-rich oil-induced peritoneal exudate cells.

5) Ultrastructural analysis of antigen localization by means of autoradiography (isotopically labelled antigen) or cytochemistry (peroxidase coupled or direct use).

6) Binding of lymphocytes to a macrophage receptor can be assessed both in the presence and absence of antigen. Monolayers of guinea pig macrophages are prepared by adherence of monocyte-macrophages from oil-induced peritoneal exudates to Lab-tek tissue culture chamber slides. After removal of non-adherent cells, 7×10^{6} lymphocytes are added to either control or antigen pulsed monolayers for varying periods of time, after which the non-macrophage

bound lymphocytes are removed by washing and the monolayer fixed with 1% gluteraldehyde for light or electron microscopic analysis.

7) The uptake and fate in culture of soluble protein antigens is being studied functionally by metabolism and cellular localization.

8) Assay of the mixed leukocyte reaction (MLR) by assay of ^{3}H-TdR incorporation occurring when cells from a variety of strain 2 and 13 guinea pig lymphoid tissues are mixed and cultured for 5-7 days in vitro.

Major Findings:

It is generally recognized that macrophages facilitate a variety of in vitro thymic-dependent immunological phenomena in the mouse, guinea pig, and man. We have analyzed the cellular and molecular events which underlie their function. Antigen specific activation of mediator production and clonal expansion of T-lymphocytes requires the presence of macrophages, and the effects of macrophages in such activation extend beyond maintenance of in vitro culture conditions. Indeed in the guinea pig, one can clearly demonstrate that recognition of soluble protein antigens by the T lymphocyte requires an initial uptake of antigen by the macrophges.

By contrast the role of macrophages in stimulation of B lymphocytes by specific antigen differs significantly from that played for T cell activation. Macrophages suppress proliferation of B cell populations depleted of T lymphocytes. Indeed proliferation of B cells appears to be more specifically under the influence of soluble factors elaborated by T cells subsequent to antigen-specific activation.

The biologic constraints inherent in a two cell immunogenic recognition unit are clear. It is necessary to define both the cellular and immunological specificity of this requirement for macrophages. In the simplest experiments, the proliferative capacity of populations of lymphoid cells depleted of macrophages are restored by adherent cells but not by lymphocytes, granulocytes, or fibroblasts. This requirement for macrophages in T cell antigen recognition is not dependent on the immune state of the animal from which the macrophage was obtained, although macrophages and lymphocytes must share genetic identity at some portion of the major histocompatibility complex (MHC) for successful detection of the antigenic signal borne by the macrophage.

The role of macrophages in antigen specific proliferation in the mouse has also been examined. Our data indicate that unlike the guinea pig macrophage-dependent support of the proliferation of lymph node lymphocytes is not restricted to syngeneic macrophages. Efforts to resolve this apparent paradox are currently under investigation.

Is macrophage lymphocyte interaction a general requirement for all T-cell activation or do these associations function solely in the recognition of cell bound and soluble protein antigens? In work done by our section, the response

of T-cells to PHA was shown to also require accessory cells; but unlike the macrophage requirement for soluble protein antigen recognition, their function can be replaced by fibroblasts or increased cell density.

It now becomes crucial to establish the nature of antigen uptake processes and the physical state of macrophage-associated, metabolized antigen. Studies in our section have shown that Cytochalasin B, an agent active against a variety of membrane functions and which blocks pinocytosis, does not effect uptake of immunologically relevant protein. Trypsinization of macrophages pulsed with antigen at 4° removes all immunological activity without altering the ability of such cells to take up new antigens. If, however, trypsinization is delayed by prior culture, pulsed macrophages become progressively resistant to the effects of trypsinization such that by 120 minutes the macrophages immunologic potential is unaltered.

Macrophage lymphocyte interaction may occur either indirectly via the secretion of antigen complexed to soluble macrophage factors cytophilic for lymphocytes or via direct cell interaction. Our section has shown at least two types of physical interactions to take place. The first occurs rapidly, is independent of the presence of antigens, and is without immunologic commitment. This step requires active macrophage, metabolism, divalent cations, and involves a trypsin sensitive macrophage site. This binding does not distinguish T or B cells and is reversible such that at any given time binding represents an equilibrium between cellular association and dissociation. When this step has brought specifically immune lymphocytes into apposition with antigen bearing macrophages, a second type of binding results. This latter phenomenon is dependent upon the presence of antigen and a sharing on lymphocyte and macrophage of histocompatibility linked gene products. This association is not easily reversed and eventuates in proliferation of the bound lymphocyte.

The use of antigens under control of dominant H-linked genes and specific alloantisera which recognize cell associated antigenic determinants which are also products of H-linked genes provide useful biological probes of the function and cellular location of molecules which play critical but incompletely defined roles in the T lymphocyte antigen selection or activation process. Alloantisera block the antigen response controlled by an IR gene linked to the gene coding for the alloantigen, but had little effect on the antigen response controlled by an Ir gene which is the product of the opposite haplotype. The inhibitory activity of the alloantisera was initially presumed to be directed solely against the proliferating T-lymphocyte. However, the central role of the macrophage in the activation of T-cell proliferation suggests that an alternative site for the inhibitory effect of the alloantisera might be on the macrophage itself by blockade of the physical and/or functional interaction of the macrophage and T cell. Unfortunately experiments which attempt to define the cellular site of action of the alloantisera using combinations of antigen pulsed macrophages and T cells are not possible because efficient interaction of macrophage associated antigen and T cell is only seen when the macrophage and T cell share identity of gene products linked to the guinea pig MHC.

There are, however, a number of special conditions in which a macrophage may "paradoxically" induce allogeneic lymphocyte proliferation. These circumstances are rather revealing situations, in that they clearly shown that the failure to recognize soluble protein antigens bound to allogeneic macrophages is not a primary inability of such cells to present signals but may instead reflect a basic feature of antigen handling by macrophages.

Recently our lab has shown guinea pig macrophages to be very effective stimulators of allogeneic lymphocyte proliferation. Thus soluble proteins may be displayed in an immunologically relevant fashion only on syngeneic macrophages; whereas in the MLR, it is the histoincompatible macrophage that induces T cell proliferation, again suggesting that the critical difference lies in the display of alloantigen as opposed to that of foreign proteins.

In another model system, chemical alteration of the macrophage depleted lymphocyte or macrophage plasma membrane glycoproteins by treatment with either sodium metaperiodate or neuraminidase galactose oxidase generates free aldehyde groups on terminal sugar moieties. Subsequent and rather massive lymphocyte proliferation will occur if and only if modified lymphocyte is combined with unmodified macrophages or modified macrophages are combined with unmodified lymphocytes. Moreover, proliferation will occur irrespectively of the histocompatibility difference between the macrophage and lymphocyte. These latter observations have led us to suggest that immune recognition may in fact consist of at least two separate but linked events. The first phase of this interaction involves the selective binding of antigen specific T cells by macrophages bearing that antigen. The second or "activation" phase of macrophage lymphocyte interaction is a relatively nonspecific event which follows as a consequence of stabilization of physical macrophage lymphocyte interaction initiated by the antigen specific selection process.

Based on the results of studies pursued in our section, models for the role of the macrophage in genetic control of T cell responses to soluble protein antigens must consider: 1) an antigen specific receptor on the T cell, 2) cellular interaction structures identical to or closely linked to the alloantigens, 3) the IR gene product functioning either as the specific T cell receptor or as an auxillary antigen recognition molecule on the macrophage and/or T cell, 4) antigen bound to metabilocally intact macrophages in a form resistant to proteolytic treatment and located in a cellular site not available to specific antibody, and 5) a macrophage associated nonspecific membrane trigger site or secretory product as suggested by the activation of aldehyde treated lymphocytes by normal macrophages.

At present, our section favors the hypothesis which states that a given macrophage's repertoire of IR gene products function to specify determinant selection. Possible mechanisms by which the interaction of antigen with the IR gene product could select determinant specificity are as follows:

1) Enzymatic - The IR gene product is an enzyme of relative broad specificity such as a protease which destroys only those polypeptide sequence towards which it has reactivity.

2) Conformational - The IR gene product alters the tertiary structure of the antigen itself so as to display selected regions of the polypeptide to the T cell.

3) Receptor Presentation - This would require that certain similar existing amino acid sequences or conformations in a complex antigen be recognized by IR gene products. Such receptor molecules would possess specific binding properties akin to that of the binding site of a proteolytic enzyme but would differ from the enzymatic model in that no selective degradation of the antigen occurs. The function of such class specific antigen binding molecules would be to orient or display those regions of the polypeptide antigen not bound to the receptor.

Genetic control of immune responsiveness to both synthetic and natural polypeptide antigens has been found in experimental animals. Unfortunately absolute demonstration of such phenomenon in man has not been possible because of the limited number of ethical antigens which can be used in man and their lack of defined physical structure. One antigen which might be of significant potential in such studies is insulin. Beginning with studies in inbred Strain 2 and 13, we have found that cellular and humoral immune responsiveness of distinct regions of the molecule are under control of histocompatibility-linked genes. Strain 2 guinea pig T cells (as measured by proliferation or T cell help) recognize a determinant consisting of amino acids 6-11 of the A chain (α loop) while Strain 13 guinea pig T cells recognize a region in the insulin B chain. Nonetheless, the determinants recognized by antibody generated in both strains predominantly recognize shared determinants. Recently, we have initiated studies in insulin requiring diabetics without allergic complications. Of 10 patients requiring daily insulin maintenance none exhibited in vitro antigen induced T cell proliferation. At this time it is unclear whether this is a "true" non-responsiveness or tolerization due to the mode of daily insulin therapy, i.e. development of suppressor cells, sequestation or high-dose tolerance. Studies are in progress to explore this.

Significance to Biomedical Research and the Program of the Institute:

The characterization of the immune responsiveness of thymus-derived lymphocytes should provide a useful system for study of the role of the cellular immune effector cells in immunologic surveillance and their contribution to the humoral limb of hist defenses. Tumor immunity and transplantation immunity have been shown to rely to a great extent upon mechanisms of cellular immune function such as cytotoxicity and elaboration of other so-called "soluble mediators". A more fundamental understanding of the immunobiology of these crucial events and morphologic correlates may provide new insights into immune competence in man and information on the mechanisms of cellular regulation of

immune responsiveness. Potential approaches to therapeutic manipulation of such events would be of immediate clinical relevance.

Proposed Course:

1) Characterize the role of the macrophage in in vivo and in vitro initiation of cell-mediated immunity in experimental animals.

2) Characterize the localization and fate of macrophage-associated antigen in in vitro antigen-mediated lymphocyte proliferation.

3) Explore the role of histocompatibility and genetic regulation in cell-cell interactions required for expression of cellular immunity.

4) Determine if genetic control of the immune response to insulin exists in man.

Publications:

1. Ben-Sasson, S.Z. and Rosenthal, A.S.: Temporal dependence of the inhibition by cytochalasin B of antigen initiated migration inhibition factor production. J. Immunol. 115: 1140-1142, 1975.

2. Ellner, J.J. and Rosenthal, A.S.: Quantitative and immunologic aspects of the handling of 2,4 dinitrophenyl guinea pig albumin by macrophages. J. Immunol. 114: 1563-1569, 1975.

3. Gallin, E.K., Wiederhold, M.L., Lipsky, P.E., and Rosenthal, A.S.: Spontaneous and induced membrane hyperpolarizations in macrophages. J. Cell. Physiol. 86: 653-662, 1975.

4. Greineder, D.K. and Rosenthal, A.S.: Macrophage activation of allogeneic lymphocyte proliferation in the guinea pig mixed leukocyte culture. J. Immunol. 114: 1541-1547, 1975.

5. Greineder, D.K. and Rosenthal, A.S.: The requirement for macrophage-lymphocyte interaction in T lymphocyte proliferation induced by generation of aldehydes on cell membranes. J. Immunol. 115: 932-938, 1975.

6. Lipsky, P.E., Hardin, J.A., Schour, L., and Plotz, P.H.: Spontaneous peritonitis and systemic lupus erythematosus. J. Am. Med. Assoc. 232: 929-931, 1975.

7. Lipsky, P.E. and Rosenthal, A.S.: Macrophage-lymphocyte interaction: antigen-independent binding of guinea pig lymph node lymphocytes by macrophages. J. Immunol. 115: 440-445, 1975.

8. Rosenthal, A.S. (Editor): Immune Recognition. New York, Academic Press, 1975, 855 pp.

9. Rosenthal, A.S. and Balow, J.E.: Mechanisms of glucocorticoid suppression of cell-mediated immunity. In van Furth, R. (Ed.): Mononuclear Phagocytes in Immunity, Infection and Pathology. London, Blackwell Scientific Publications, Ltd., 1975, pp. 701-710.

10. Rosenthal, A.S., Blake, J.T., and Lipsky, P.E.: Inhibition of macrophage-lymphocyte interaction by cytochalasin B during antigen recognition by T lymphocytes. J. Immunol. 115: 1135-1139, 1975.

11. Rosenthal, A.S., Lipsky, P.E., and Shevach, E.M.: Macrophage-lymphocyte interaction and antigen recognition. Fed. Proc. 34: 1743-1748, 1975.

12. Rosenthal, A.S., Lipsky, P.E., and Shevach, E.M.: Morphological and functional correlates of macrophage-lymphocyte interaction. In van Furth, R. (Ed.): Mononuclear Phagocytes in Immunity, Infection and Pathology. London, Blackwell Scientific Publications, Ltd., 1975, pp. 813-825.

13. West, B.C. and Rosenthal, A.S.: Isolation and partial characterization of human eosinophil granules: comparison to neutrophils. Am. J. Pathol. 81: 575-588, 1975.

14. Ellner, J.J., Lipsky, P.E., and Rosenthal, A.S.: Phytohemagglutinin-induced proliferation of guinea pig thymus-derived lymphocytes. II. Accessory cell function. J. Immunol. 116: 876-880, 1976.

15. Lipsky, P.E., Ellner, J.J., and Rosenthal, A.S.: Phytohemagglutinin-induced proliferation of guinea pig thymus-derived lymphocytes. I. Accessory cell dependence. J. Immunol. 116: 868-875, 1976.

16. Rosenthal, A.S., Blake, J.T., Ellner, J.J., Greineder, D.K., and Lipsky, P.E.: The macrophage function in antigen recognition by T lymphocytes. In Nelson, D.S. (Ed.): Immunobiology of the Macrophage. New York, Academic Press, 1976, pp. 131-160.

17. Rosenthal, A.S. and Shevach, E.M.: Macrophage-lymphocyte interaction: cellular basis for genetic control of T lymphocyte antigen recognition. In Katz, D.H. and Benacerraf, B. (Eds.): The Role of Products of the Histocompatibility Gene Complex in Immune Responses. New York, Academic Press, 1976, pp. 335-350.

18. Lipsky, P.E., Ellner, J.J., and Rosenthal, A.S.: Dissection of the role of accessory cells in phytohemagglutinin-induced T lymphocyte proliferation. In Eijsvoogel, V. (Ed.): Tenth Leukocyte Culture Conference. New York, Academic Press, in press.

19. Rosenthal, A.S. and Shevach, E.M.: Function of macrophages in T lymphocyte antigen recognition. In Weigle, W. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00050-06 LCI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Clinical Studies of Complement Abnormalities of Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. Frank	Head, Clinical Immunology Section	LCI	NIAID
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	J. Gadek	Clinical Associate	"	"
	S. W. Hosea	Clinical Associate	"	"
	T. Gaither	Research Biologist	"	"
	R. Reinhart	Biol. Lab. Tech. (BioChem.)	"	"

COOPERATING UNITS (if any)

D. W. Alling (OSD, NIAID, NIH)
R. Sherins (NICHHD, NIH)
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LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3-6/12	1-11/12	1-8/12

SUMMARY OF WORK (200 words or less - underline keywords)

This year, major emphasis has been placed upon the development of new therapy for patients with hereditary angioedema. A drug was sought which would be relatively nontoxic, which could be given to both men and women and which would control symptoms of the disease. For a number of reasons, a drug named Danazol produced by Sterling Winthrop Laboratories was chosen for study. A double blind study was completed of the use of this drug in both men and women. The drug was shown to be highly effective in the treatment of hereditary angioedema. Of equal interest is the fact that the drug was shown to correct the genetically controlled biochemical abnormality, returning C1 esterase inhibitor and C4 levels to normal in most patients on therapy. It would appear that this drug will be the most useful available method for the treatment of this disease. A second project was begun during this period concerning the use of purified preparations of the C1 esterase inhibitor prepared by the American Red Cross for infusion therapy of patients with hereditary angioedema during acute attacks. These studies are only starting at the present time, and it is not yet known whether this will be effective therapy or not.

Project Description:

Objectives:

The general objective of this program has been to define the role of complement in human illness and to develop better methods of therapy for immune damage mediated by complement. As in the past year major emphasis was placed on our studies with patients with hereditary angioedema. The group of patients is growing rapidly and we are now following at least forty families with patients with this disorder.

Methods Employed:

Purified complement components are prepared, and antisera to various complement components and component inhibitors are used for estimation of component levels in human sera and other body fluids. Double blind therapeutic studies are employed to test new methods of therapy in patients. Patients on experimental drug protocols are followed to determine signs of toxicity. In 1972, our group reported the first double blind study of treatment of patients with hereditary angioedema with Epsilon amino caproic acid (EACA). We showed that this antifibrinolytic drug was effective in therapy of this disease and became the major group following patients on this method of therapy. We found that this drug was fairly effective in treating these patients. However, a group of patients had significant side-effects including neuromuscular weakness and muscle aches and pains. Other drugs were studied as therapy for this illness in a double blind fashion and did not prove to be effective. In the past year, we have studied a newly developed, very weak androgen, Danazol. This drug, a gonadotropin inhibitor, has been developed for treatment of endometriosis in women and as an oral contraceptive. It is thought to have minimal toxicity aside from its hormonal mediated effects. Its androgenic activity is about 1/100 that of methyltestosterone. During this year, a double blind study of Danazol therapy has been continued. A total of nine patients were studied on this drug--five women and four men. The drug was shown to be extremely effective in therapy of hereditary angioedema. In fact, in approximately 88 double blind courses of drug or placebo of which about half were courses of drug, only one attack was seen. This is in contrast to 41 attacks of hereditary angioedema seen in this group of patients during 44 courses of placebo therapy. When sera from these patients were examined for the level of the C1 esterase inhibitor and C4, a striking observation was made. The C1 esterase inhibitor, a protein whose levels are depressed in patients with this disease due to a genetic abnormality in their ability to synthesize the protein, was found to show striking increases. In fact, in more than half of the patients the level of this protein increased to normal or supranormal levels. Concomitant with the increase in the C1 esterase inhibitor the C4 level rose an average of fifteen-fold, returning to normal in most of the patients. Thus, this drug provides not only effective therapy of hereditary angioedema but reverses a genetically controlled biochemical abnormality. This is of great interest, and attention will be focused next year on attempting to identify the mechanism of action of this drug. This may give us a further more basic understanding of this disease.

Another phase in our study of hereditary angioedema was initiated during the past year. There is no good therapy for acute attacks of this disease. All therapy thus far has been essentially prophylactic; i.e., directed at preventing attacks. This year, in cooperation with the American Red Cross, we have begun the study of the infusion of the purified C1 esterase inhibitor protein into patients with hereditary angioedema. This study is still in its infancy, and no data is yet available. The American Red Cross is preparing the protein for us, and it will be available in a tenfold concentrated form over its level in normal plasma. Last year, we showed that plasma infusions could provide effective prophylaxis of this disease in patients who are going to undergo dental surgery. It is hoped that by infusing the C1 esterase inhibitor free of complement proteins, we will be able to treat the disease without the theoretical complication of transciently making it worse due to the infusion of complement proteins. The complement components are separated from the C1 esterase inhibitor during the preparation of this protein. We plan to infuse the equivalent of two units of fresh frozen plasma into patients at a time when they are free of symptoms. We then plan to follow the level of the C1 esterase inhibitor protein as well as the level of C4 for a period of one to two weeks after the infusion. We will determine whether the level of the C1 esterase inhibitor rises and whether this in turn causes the C4 level to rise to normal. If these objectives are reached, we will extend the study to the treatment of patients with acute attacks of hereditary angioedema.

Significance to Biomedical Research and the Program of the Institute

In the past, hereditary angioedema has been considered to be a very rare disease. With each year, the finding of increasing numbers of patients suggests that this disease is by no means as uncommon as previously suspected. These patients are very ill with their disease and experience mortality approaching 25 or 30% from life-threatening attacks of angioedema. Thus, the development of effective treatment is in itself a useful objective. By study of this disease, we are learning more about how serum mediators, including complement, influence diseases in man. The present studies may, in addition, allow us to learn more about protein synthesis in man and about the genetic basis of this interesting disease. We also plan to study the usefulness of Danazol in a variety of other situations in which genetic abnormalities exist in the synthesis of serum inhibitor proteins.

Publications:

1. Jaffe, C. J., Atkinson, J. P., Gelfand, J. A., and Frank, M. M.: Hereditary angioedema: The use of fresh frozen plasma for prophylaxis in patients undergoing oral surgery. J. Allergy and Clin. Immunol. 55: 386-393, 1975.

2. Frank, M. M., Gelfand, J. A., and Atkinson, J. P.: Hereditary Angioedema: The clinical syndrome and its management. Combined Clinical Staff Conference. Ann. Intern. Med. 84: 580-94, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00051-05 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Interactions of Pseudomonas Bacteria, Opsonins, and Alveolar Macrophages in the Respiratory Tract (Revised Title)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Y. Reynolds, Head, Bacterial Diseases Section, LCI, NIAID

OTHER: J. A. Kazmierowski, Clinical Associate, LCI, NIAID
R. P. Aduan, Clinical Associate, LCI, NIAID

COOPERATING UNITS (if any)
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Z01 AI 02407-02 PB
R. G. Graw, Jr. (Pediatric Oncology Branch, NCI, NIH) Z01 CM 06820-03 PO

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SECTION
Bacterial Diseases Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2- 5/12	1- 5/12	1

SUMMARY OF WORK (200 words or less - underline keywords)

The general area of pulmonary host defenses and nosocomial infections is being investigated, using Pseudomonas pneumonia as the prototype infection because of its frequent occurrence in immunosuppressed patients who are most susceptible to opportunistic microorganisms. Initial work has been done in rabbits and now extended to humans to explore ways of manipulating antibody production in the respiratory tract and to assess the effectiveness of these antibodies in promoting phagocytosis and killing by alveolar macrophages. IgG was found to be the principal opsonin in the lower respiratory tract secretions, sampled by bronchoalveolar lavage. IgA, although present with agglutinating and opsonic activity, was less effective than IgG; IgM was ineffective. The results correlate with the presence of specific surface receptors of alveolar macrophages which determine what these cells can fix to their surface and subsequently internalize by phagocytosis. The C_3 complement receptor on these cells may augment the response but its function has not been explored completely.

Project Description:

Objectives:

Pseudomonas aeruginosa is a serious opportunistic bacterial pathogen for patients with impaired host immunity due to hematological malignancies or from immunosuppressive therapy for various neoplastic or other systemic diseases. Also, patients with extensive burns or structural lung diseases, particularly those who require assisted ventilation treatment, are at high risk to develop Pseudomonas infections. Pseudomonas colonization of the respiratory tract and subsequent pneumonia is particularly troublesome because the patient's response to antibiotics is often not dramatic and the microorganisms may not be eradicated. Another approach to improve therapy of these pulmonary infections is that of immunological prophylaxis through the development of specific anti-Pseudomonas antibodies in serum and in the respiratory tract. These antibodies, particularly those with opsonic antibody activity, may help the lungs' cellular defense mechanism (pulmonary macrophages) by enhancing phagocytosis and killing of Pseudomonas. Replacement of phagocytic cells (granulocytes) may be essential in leukopenic hosts as well.

The initial phases were conducted in experimental animals and have expanded now into a more general assessment of pulmonary host defenses which includes several human experimental protocols, employing Pseudomonas aeruginosa vaccine (Parke-Davis, Inc. BB-IND 185), and in the study of an immunosuppressed canine model of experimental pneumonia. Generally, in the past year, our studies have concentrated more on protein and cellular elements in the human and primate respiratory tracts. Broncho-alveolar lavage performed in conjunction with diagnostic fiberoptic bronchoscopy has been used. Studies initially were directed to 1) establishing normal values for various immunoglobulins and other proteins in respiratory lavage fluid, 2) identifying the surface membrane receptors on alveolar macrophages, and 3) assessing the functional capacity of IgG and IgA opsonins to enhance alveolar macrophage phagocytosis of pseudomonas. Most recently, studies have been extended to a variety of patients with chronic interstitial lung diseases to see if these methods will help elucidate the pathophysiology of such disorders. Work to characterize lymphocyte subpopulations recovered in lung washings has utilized Rhesus monkeys.

Methods Employed:

Animals are vaccinated with a variety of monovalent and polyvalent whole-cell or lipopolysaccharide Pseudomonas vaccines to observe the development of antibody in serum and bronchial secretions. Agglutinating antibodies, total titers and 2-mercaptoethanol resistant (IgG) titers, are measured. Isolation and purification of antibodies from serum and bronchial secretions employs gel filtration and ion exchange chromatography, sucrose density gradient ultracentrifugation, zone electrophoresis and other standard concentration and separation techniques. Macrophages, established in short-term cell monolayer cultures with well described techniques, are challenged with ^{14}C labeled bacteria, which have been previously opsonized; subsequent

phagocytosis is measured with liquid scintillation spectroscopy and bacterial killing is assayed with standard agar pour plate cultures. Bronchial secretion proteins are quantitated by immunodiffusion precipitation methods. In addition, cellular immunity is evaluated by skin testing (histology) and in vitro culture of lymphocytes. Lymphocytes are stimulated to produce lymphokines (migration inhibition factor) and the migration of alveolar macrophages is studied.

Major Findings:

The type of specific anti-Pseudomonas antibody induced in the lungs or bronchial secretions depends upon the route of vaccination used - intramuscular (IM) or intranasal. Following parenteral (IM) immunization, IgG agglutinative and opsonic antibody is present in lung secretions which diffuses into the lung from the blood; in contrast, intranasal immunization produces secretory IgA and secretory IgG antibodies in addition to a modest systemic or serum antibody response. These bronchial antibodies have been isolated and characterized. However, IgG seems to be the more important opsonic antibody in the on the basis of phagocytic and killing studies done with in vitro alveolar macrophages and ^{14}C labeled Pseudomonas organisms. These animal studies comparing the relative activity of IgG and IgA opsonins were extended to humans.

First, specific cell membrane surface receptors for immunoglobulin and complement components were identified by rosette formation on in vitro cultured alveolar macrophages obtained from 24 humans. Respiratory macrophages have easily identified receptors for IgG and the C3b fragment of the third component of complement. A macrophage receptor for the C3d fragment was detected only when purified human complement components were used to form erythrocyte-antibody-C3 immune complexes but was not detected when whole human serum was used as the source of complement. No IgM cell receptor was identified. Thus, with respect to membrane receptors, alveolar macrophages resemble peripheral blood monocytes. These studies in addition emphasize the importance of using a variety of immune reagents, especially the use of human reagents, to determine correctly these cell receptors.

Next, specific secretory IgA (sIgA) and IgG antibodies were compared for activity to promote phagocytosis of viable Pseudomonas aeruginosa by human alveolar macrophages. Prior identification of cell membrane surface receptors was most helpful in completing these experiments. Macrophages were obtained by lung lavage of normal adult smoker and nonsmoker volunteers and were maintained as in vitro cell monolayers. Both immune sIgA and IgG agglutinating antibodies were demonstrated to coat and opsonize viable bacteria, whereas similar nonimmune immunoglobulin preparations did not. When alveolar macrophages were challenged with viable opsonized ^{14}C-labeled Pseudomonas, IgG-reacted bacteria were ingested better and killed more readily than sIgA-opsonized organisms. Phagocytic responses were not significantly different between macrophages obtained from smokers and nonsmokers. Although sIgA and IgG antibodies can be found in respiratory secretions and both are undoubtedly

important in pulmonary host defense, IgG opsonic antibody was superior in enhancing the uptake of Pseudomonas by in vitro-cultured alveolar macrophages. It may be the more important respiratory antibody for certain bacterial infections.

With respect to lymphocytes recovered in broncho-alveolar lavage, at least two populations of lymphocytes can be identified in primate lung washings, one forming rosettes with SRBC and another forming rosettes with erythrocyte-antibody-complement complexes. The response to mitogens by cells from lavage fluid is similar to spleen and blood lymphocytes. The distribution of lymphocytes in pulmonary lavage fluid is similar to that found in the peripheral blood. The identity of lymphocyte popoulations in respiratory fluids has relevance in understanding the immunological capabilities of the primate respiratory tract.

Significance to Biomedical Research and the Program of the Institute:

Pseudomonas infections in patients with altered immunity or with structural lung disease is a major infectious disease problem which caused a high number of acquired infections in hospitalized patients. As yet little attention has been given to the induction of protective antibodies in the respiratory tract and to the subsequent control of pulmonary colonization by Pseudomonas. Certainly, from the experimental animal studies described, it is possible to obtain specific bronchial antibody but to do so, immunization must be prolonged enough to ensure development of IgG antibody. In the presence of the antibody which is available to opsonize Pseudomonas organisms, pulmonary macrophages in vitro are efficient in ingesting and killing these organisms.

Alveolar macrophages are the most abundant respiratory tract cell recovered in bronchial lavage fluid from normal lung tissue. These cells are the principal phagocytes of the lung and serve to clear small inhaled particles, including bacteria, which are deposited in the terminal airways (alveoli). Presumably, they are aided in this function by the presence of the specific membrane receptors for immunoglobulin and complement that have been identified on their surfaces. It was interesting to correlate alveolar receptor identify with actual constituents in the normal alveolar environment to arrive at some estimate of the physiologic significance of these cell receptors. For example, in the normal respiratory tract, IgG and IgA are the principal immunoglobulins present. IgM is usually absent and complement components are present in low amounts. It was demonstrated that the Pseudomonas organisms opsonized with IgG can be ingested by alveolar macrophages and Pseudomonas organisms coated with secretory IgA cannot. Presumably this is a function of the presence of the IgG membrane receptor and the absence of an IgA receptor. Moreover, IgM-coated Pseudomonas organisms are not phagocytosed by alveolar macrophages and, indeed, these studies have not demonstrated the presence of an IgM receptor. During an inflammatory reaction in the lung, it might be expected that serum proteins, including complement, can diffuse into the inflamed tissue site and complement receptors may be of great importance under these conditions.

In summary, an important aspect of these studies [illegible] attempt to identify the immunoglobulin class that provides the most efficient opsonic antibody for certain bacterial infections of the lung. This finding could influence the route of immunization for bacterial vaccination or the products used for passive antibody administration.

Proposed Course:

1. Patients considered to be at high risk for Pseudomonas pulmonary infections, will be vaccinated with the Parke, Davis and Co., polyvalent Pseudomonas vaccine (Pseudogen) to determine the optimal vaccination regimen necessary to produce specific IgG bronchial antibodies. Observations concerning the protective value of these antibodies will be made. Intranasal or possible intrabronchial immunization will be done in patients with chronic lung diseases who are at increased risk for Pseudomonas pneumonia in an attempt to maximize respiratory antibodies.

2. Continuation of the experimental pneumonia model in neutropenic and immunosuppressed dogs. Studies evaluating passive immunization with IgG agglutinating antibody are in progress.

3. Studies with human respiratory macrophages and opsonic antibodies, similar to the animals experiments, will be continued. Further analysis of the cellular and protein milieu of the lower lung is underway. Patients and normal volunteers are undergoing bronchial lavage via transnasal fiberoptic bronchoscopy to obtain cells and protein components from the lower respiratory tract. Studies will be extended from normal subjects to those with a variety of chronic interstitial lung diseases to see if cellular and protein analysis of broncho-alveolar lavage can improve diagnosis and hopefully take the place of more invasive diagnostic procedures such as open lung biopsy.

Publications:

1. Reynolds, N. Y., Levine, A. S., Wood, R. E., Zierdt, C. H., Dale, D. C., and Pennington, J. E.: Pseudomonas aeruginosa infections: Persisting problems and current research to find new therapies. NIH Combined Clinical Staff Conference, Ann. Intern. Med., 82: 819-831, 1975.

2. Reynolds, H. Y., Atkinson, J. P., Newball, H. H., and Frank, M. M.: Receptors for immunoglobulin and complement on human alveolar macrophages. J. Immunol., 114: 1813-1819, 1975.

3. Reynolds, H. Y., Kazmierowski, J. A., and Newball, H. H.: Specificity of opsonic antibodies to enhance phagocytosis of Pseudomonas aeruginosa by human alveolar macrophages obtained from cigarette smokers and nonsmokers. J. Clin. Invest., 56: 376-385, 1975.

4. Pennington, J. E., Dale, D. C., Reynolds, H. Y., and MacLowry, J.: Gentamicin sulfate pharmacokinetics: Lower levels of gentamicin in blood during fever. J. Infect. Dis., 132: 270-275, 1975.

5. Pennington, J. E., Reynolds, H. Y., Wood, R. E., Robinson, R. A., and Levine, A. S.: Use of a Pseudomonas aeruginosa vaccine in patients with acute leukemia and cystic fibrosis. Am. J. Med. 58: 629-636, 1975.

6. Dale, D. C., Reynolds, H. Y., Pennington, J. E., Elin, R. J., and Herzig, G. P.: Experimental pseudomonas pneumonia in leukopenic dogs: comparison of therapy with antibiotics and granulocyte transfusions. Blood. 47: 869-876, 1976.

7. Kazmierowski, J. A., Fauci, A. S. and Reynolds, H. Y.: Characterization of lymphocytes in bronchial lavage fluid from monkeys. J. Immunol. 116: 615-618, 1976.

8. Kazmierowski, J. A., Elin, R. J., Reynolds, H. Y., Durbin, W. A., and Wolff, S. M.: Chediak-Higashi syndrome: reversal of increased susceptibility to infection by bone marrow transplantation. Blood. 47: 555-559, 1976.

9. Pennington, J. E.: Kinetics of penetration and clearance of antibiotics in respiratory secretions. In Kirkpatrick, C. H. and Reynolds, H. Y. (Vol. Eds.): Immunologic and Infectious Reactions in the Lung, Vol. 1 Lung Biology in Health and Disease. Claude Lenfant, Exec. Ed. New York, Marcel Dekker, Inc., 1976, Chap. 17, pp. 355-374.

10. Reynolds, H. Y., Kazmierowski, J. A., and Dale, D. C.: Changes in the composition of canine respiratory cells following irradiation or drug immunosuppression. Pro. Soc. Expl. Biol. Med. 151: 756-761, 1976.

11. Reynolds, H. Y. and Newball, H. H.: Milieu of the human respiratory tract: Analysis of cells, proteins, complement and lipids obtained from the lungs by bronchial lavage. In Kirkpatrick, C. H. and Reynolds, H. Y. (Eds.): Immunologic and Infectious Reactions in the Lung. New York, N.Y., M. Dekker, Inc., 1976, p. 3-27.

12. Reynolds, H. Y.: The interaction of bacteria, antibodies, macrophages, and lymphocytes in the lung following immunization with Pseudomonas antigens. In Kirkpatrick, C. H. and Reynolds, H. Y. (Eds.): Immunologic and Infectious Reactions in the Lung. New York, M. Dekker, Inc., 1976, p. 143-159.

13. Crystal, R. G., Fulmer, J. D., Moss, M. L., Roberts, W. C., Line, B. R. and Reynolds, H. Y.: Fibrotic Lung Disease. NIH Combined Clinical Staff Conference. Ann. Intern. Med., In press, 1976.

14. Kazmierowski, J. A., Durbin, W. A., and Reynolds, H. Y.: Kinetics of immunoglobulin transport into canine bronchial secretions. Proc. Soc. Expl. Biol. Med., In press., 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00054-04 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Etiology and Pathogenesis of Viral Gastrointestinal and Respiratory Tract Infections in Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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ABSTRACT OF WORK (200 words or less - underline keywords)

The pathogenesis of viral gastrointestinal and respiratory infections in man, with particular emphasis on cell mediated immunity is being examined. Influenza infections result in virus specific cell mediated immune responses as measured by virus specific in vitro lymphocyte blastogenesis and lymphocytotoxicity: These responses may be mediated by hemagglutinin receptors on human lymphocytes. A new interferon inducer, PICLC, appeared to be non-toxic when administered intranasally to volunteers, but had no effect on experimentally induced rhinovirus infection. Viral gastroenteritis induced by the parvovirus-like agents appeared to induce the production of local antibodies to the agents in gastrointestinal secretions. Human parvoviruses (adenovirus-associated viruses) are potentiated in vitro by tissue culture cells chronically infected with herpes simplex virus or transformed by adenovirus.

Objectives:

1) To define the biologic, immunologic, and epidemiologic properties of the etiologic agents of viral gastroenteritis in man.

2) To develop *in vitro* culture systems and/or laboratory animal models with which to study these agents.

3) To investigate the pathogenesis of and host response to viral infections of the gastrointestinal and respiratory tracts in man. Experimentally induced disease in normal volunteers, as well as naturally occurring cases, are studied.

4) To examine the possible roles of viral agents in acute and chronic gastrointestinal and respiratory tract diseases of unknown etiology.

5) To devise control measures based on the above information, with particular emphasis on the use of antiviral compounds.

6) To study the biological and immunological properties of parvoviruses, with emphasis on adenovirus-associated viruses and their potential role in human disease.

Methods Employed:

1) Source of Viral Agents: Viral agents have been obtained from naturally occurring outbreaks of viral gastroenteritis, and have been partially characterized. These are maintained as infectious suspensions in veal infusion broth and serve as infectious inocula and sources of antigen. Respiratory tract viruses are isolated in tissue and organ culture from naturally occurring cases. After characterization, they are grown in large quantities to serve as standard sources of the agent for laboratory and volunteer studies.

2) *In Vitro* Culture Systems: Standard tissue cultures or suckling mice are employed for the isolation of conventional viral agents. Since certain fastidious agents replicate only in organ culture, human fetal intestinal and tracheal organ cultures are employed for the detection of new or occult agents. The organ cultures are examined with light, fluorescence and electron microscopy, and the explants are tested for viral interference, metabolic alterations, and enzyme production.

3) Clinical Studies

a) Volunteer Studies: After rigorous safety tests of the inocula, normal volunteers are screened for appropriate serum antibody status and are infected via oral or nasopharyngeal administration of these viruses. The volunteers are followed closely for clinical changes, and samples of blood, stool, respiratory secretions, along with selected biopsies, are obtained.

b) Naturally Occurring Cases: Similar studies are performed on outpatients and hospitalized patients referred by local physicians or the infectious disease consultation service of the LCI. In collaboration with Dr. A. S. Levy, mucosal biopsy specimens, stool samples and sera from patients with ulcerative colitis and regional enteritis are provided for viral studies. In collaboration with Dr. Fullmer of NHLI, lung biopsies, as well as respiratory tract secretions, from patients with chronic interstitial pulmonary disease of unknown etiology, are provided for study. Lung biopsies of acute interstitial pulmonary infiltrates in host defense-compromised patients hospitalized at the Clinical Center are provided through the consultation service of the LCI.

4) Host-Defense Studies: Serum and secretory antibody responses to viral infection are examined, employing neutralization, complement fixation, hemagglutination inhibition or fluorescent antibody assays. Cell mediated immunity is investigated by mitogenic and antigenic stimulation of lymphocyte cultures and by lymphocytotoxicity assays against virus-infected target cells. Lymphocyte subpopulations are determined by sheep red blood cell and complement receptor assays (Fauci). Nonspecific defenses such as polymorphonuclear leukocyte functions and interferon production are also examined.

5) Adenovirus-associated viruses are grown in tissue culture, purified, and detected by fluorescent antibody and complement fixation assays. These defective agents are grown in the presence of intact helper viruses (adeno and herpes viruses), and of conditionally defective mutant viruses or virus-containing cells. These studies are designed to define the characteristics of the helper functions of these agents.

Major Findings:

1) Influenza.

a) Cell mediated immune responses were further investigated in 17 normal volunteers challenged intranasally with H3N2 or HONI influenza viruses. Despite depression of blastogenic response to mitogens (reported in annual report of 74-75), a blastogenic response to homologous, purified, inactivated influenza antigens was noted 2 to 4 weeks after challenge. This response was correlated with the level of hemagglutinin-inhibiting (HI) circulating antibody (P=.007) but not with the level of neuraminidase-inhibiting (NI) antibody (P=.57). The blastogenic response was specific for H3 antigen and did not cross react with HO, suggesting that infection with unrelated influenza viruses does not confer cell mediated immunity to antigenically distinct (eg. pandemic) strains.

b) An assay of direct lymphocytotoxic activity against 51Cr labeled tissue culture cells infected with influenza viruses was established. Low levels of cytotoxicity in lymphocyte cultures from volunteers were detected as early as 6 days after infection with HONI viruses, prior to the development of detectable serum HI antibody. Cytotoxicity was also examined in

a similar assay in C 3H/HeJ and C 57 Bl. 10 Sn mice infected intranasally with the PR-8 influenza strain, which resulted in histologic evidence of pneumonia, and high titers (10^5 TCID 50/gm) of influenza virus in the lungs. Levels of cytotoxicity of splenic lymphocytes reached their peak at 9 days after infection (20-40%) and fell to 10-15% 13 days after infection. Employment of H2 histocompatible tissue culture target cells and effectors resulted in detection of higher levels of cytotoxicity early after infection (day 6); however, levels at later time points were not significantly different from those seen with H2 incompatible cells.

c) The direct interaction between lymphocytes and influenza virus was examined in vitro. Human lymphocytes, as well as monocytes and polymorphonuclear leukocytes, are agglutinated by inactivated influenza viruses, irrespective of the history of previous infection in the donor. This appears to be mediated by a hemagglutinin receptor, with reaction kinetics similar to those which occur between myxoviruses and red cells. The characterization of this receptor is currently under way.

d) In cooperation with the BOB, the first trials of inactivated influenza A/Swine vaccine were carried out in NIH and FDA employees. Employing whole virus and split product preparations at doses of 200,400 and 800 CCA units, 144 volunteers were inoculated intramuscularly. Significant systemic reactions occurred in 3%, while low grade fever was noted in another 3%. Serum antibody (HI and NI) responses will be measured 3 weeks past inoculation. Additional trials involving 300 volunteers with tri-valent A/Swine, A/Victoria, and B/Hong Kong influenza viruses are planned for mid-May.

2) Rhinovirus Infections. Studies on the pathogenesis and potential chemoprophylaxis of rhinovirus infections were begun. A new interferon inducer, poly-I-poly-C-L-lysine (PICLC) complexed with carboxymethycellulose to confer resistance to degradation by primate nucleases, was administered intranasally to 6 volunteers at doses of 0.3 mg/kg/day for 7 days without evidence of local or systemic toxicity. After development of a reproducible challenge system, 10 sero-negative volunteers received 100 TCID50 of Rhinovirus intranasally. Administration of PICLC at the above dosage for 36 hrs. prior to and 5 days after challenge did not reduce clinical symptoms nor virus shedding, when compared to a placebo preparation. Additional studies employing higher doses of PICLC, as well as other potential anti-viral agents are planned for the coming year.

3) Viral Gastroenteritis:

Local immunity to the parvovirus-like gastroenteritis agents (Norwalk, Hawaii, MC) was examined. Sufficiently pure antigens were prepared by isopycnic ultracentrifugation of stool filtrates in cesium chloride. Jejunal secretions were obtained via oral polyethylene tubes inserted in volunteers prior to and 2 weeks following experimental infection with the above agents. Aggregation of antigen by secretions, as detected by immune electron microscopy, was employed as an end point. Undiluted secretions prior to infection

demonstrated aggregation (1-2+ on a scale of 0-4+) of antigen. Convalescent secretions from volunteers who had been ill or who had serologic evidence of infection demonstrated 3 to 4+ aggregation, while there were no significant changes in secretions from volunteers who remained uninfected. Sucrose gradient analysis indicated that aggregation activity resided in 7S and 11S fractions, suggesting that both IgG and IgA may be involved in this response. Additional studies to quantitate this response are under way.

4) Parvoviruses:

To define the helper role of adenoviruses and herpesviruses in the replication of human parvoviruses, human prostate adenoma tissue culture cells, chronically infected with herpes simplex virus types 1 and 2, were grown with adeno-associated viruses (AAV) 1 and 2. Both of these cell strains potentiated AAV, with production of fluorescent stainable AAV antigen but no infectious virus. Similarly, an adenovirus transformed rat cell line provided by J. Rose, was demonstrated for the first time to potentiate AAV. Since none of these cell lines produce infectious helper virus, this system should aid greatly in defining the helper function. Isolation of such a function may aid in the in vitro cultivation of parvovirus-like agents of gastroenteritis.

5) Chronic Pulmonary and Inflammatory Bowel Disease:

Nine lung biopsies from patients with acute and chronic interstitial pulmonary disease were examined for the presence of viral agents, with the detection of CMV in one case and adenovirus 2 in another. Eight intestinal biopsies from patients with ulcerative colitis and regional enteritis are being examined for viral agents. Studies in both of these areas will concentrate on application of newly described techniques for the detection of occult agents, including the use of organ culture and immune electron microscopy.

6) Clinical Virology:

In the past year, 338 clinical specimens were examined for the presence of viral agents in support of the infectious disease consultation service of the LCI or at the request of physicians at the NIH, NNMC, or in the community. 117 (35%) of these were positive for a wide variety of viral agents. Selected viral serologies were also performed to support isolation findings or for diagnosis when viral isolations were negative.

Significance to Biomedical Research and the Program of the Institute:

Viral gastrointestinal and respiratory tract infections are the most common disease experience in American families, and affect all age groups of a broad segment of the population. As yet, there are no adequate control measures available. Identification and characterization of etiologic agents, as well as studies of the pathogenesis of these diseases in man, should form the basis for rational development of methods of prevention and treatment. Examination of the possible roles of recognized, as well as unidentified

viral agents in chronic pulmonary and G.I. illness may lead to an understanding of the etiology of those diseases. The newly developed techniques of immune electron microscopy and organ cultures, as well as volunteer experiments, should continue to provide valuable data.

Proposed Course:

Studies of the agents of viral gastroenteritis will continue. Efforts to propagate the agents in vitro in organ and tissue cultures will be pursued, and emphasis will be placed in the development of rapid, sensitive, in vitro detection methods. The pathogenesis and host response of both respiratory tract and gastrointestinal viral infections in man will continue to be examined in both experimentally induced and naturally occurring disease. The possible application of anti-viral compounds to the prophylaxis and/or therapy of viral respiratory and gastrointestinal disease will be investigated. The possible roles of viruses in chronic G. I. and pulmonary diseases will continue to be examined. The biology of human parvoviruses will be studied with emphasis of defining helper functions for possible application to the search for other occult human agents.

Publications:

1. Dolin, R., Levy, A. G., Wyatt, R. G., Thornhill, T. S. and Gardner, J. Gastroenteritis induced by the Hawaii agent: Studies of intestinal histopathology and serologic response. Am. J. Med. 59: 761-768, 1975.

2. Dolin, R. and Baron, S. Absence of detectable interferon in jejunal biopsies, aspirates, and secretions in viral gastroenteritis. Proc. Soc. Exp. Biol. Med. 50: 337-339, 1975.

3. Levy, A. G., Widerlite, L., Schwartz, C. J., Dolin, R., Blacklow, N.R., Gardner, J. O., Kimberg, D. V., and Trier, J. S. Jejunal adenylate cyclase activity in human subjects during viral gastroenteritis. Gastroenterology. 70: 321-325, 1976.

4. Dolin, R., Reichman, R. C., Fauci, A. S. Lymphocyte populations in viral gastroenteritis. Inf. Immunity. In Press.

5. Dolin, R., Richman, D. D., Murphy, B. R., and Fauci, A. S. Cell mediated immune response in influenza. J. Inf. Dis. In press.

6. Thornhill, T. S., Wyatt, R. G., Kalica , A. R., Dolin, R., Chanock, R.M. and Kapikian, A. Z. Detection by immune electron microscopy of 26-27 nm virus-like particles associated with two family outbreaks of gastroenteritis. J. Inf. Dis. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00055-04 LCI

May 1, 1975 to June 30, 19[illegible]

PROJECT (50 characters or less

Investigation of the Human Response to Man and Experimental Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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6- 4/12	4- 4/12	2

SUMMARY OF WORK (200 words or less - underline keywords)

Isolation, identification, and functional characterization of various leukocyte subpopulations have allowed us to correlate specific populations of cells with the pathophysiological phenomena in certain inflammatory and immunologically mediated diseases. Studies of the specific effects of immunosuppressive agents such as corticosteroids have delineated the differential sensitivity of these subpopulations of cells to therapeutic intervention. In extension of previous studies in this area, we have further demonstrated the differential effects of these agents on distinct subpopulations of mononuclear cells mediating cytotoxic killer cell function against various tissue targets. Such studies have clearcut clinical relevance in inflammatory and autoimmune diseases, organ transplantation, and tumor surveillance. We have demonstrated the presence of cytotoxic effector cells in human bone marrow, thus adding to our understanding of possible mechanisms of the graft versus host reaction seen in bone marrow transplantation. We have recently developed a highly reproducible hemolysis-in-gel plaque forming cell assay for the measurement of single cell antibody production following primary in vitro activation of human B lymphocytes from tonsil, bone marrow, spleen and blood. Such a model will allow in depth probing of the mechanisms of activation and suppression of human B lymphocytes towards antibody production.

Project Description:

Objectives:

1) To separate and identify purified subpopulations of lymphoid cells in man and animals in order to more accurately define their normal functional capacity as well as to study their role in such disease states as hypersensitivity vasculitis, granuloma formation, tumor and graft rejection, and the graft-versus-host reaction. In addition, to delineate the precise cellular and subcellular events associated with activation of lymphoid and mononuclear cell subpopulations in the normal immune response, and in the participation in disease activity. In particular, to characterize the sensitivity of these subpopulations of cells to various immunosuppressive agents.

2) To delineate the precise mechanisms whereby immunosuppressive agents such as corticosteroids and cytotoxic drugs affect the immune response in man and in experimental animals, specifically examining their effects on the compartmentalization as well as on the in vivo and in vitro function of the monocytes and subpopulations of lymphocytes.

3) To correlate the specific immunosuppressive effects of corticosteroids and cytotoxic drugs with the suppression of disease activity in various inflammatory and immunologically-mediated disease states in man and experimental animals, and by doing so to aim at a greater understanding and therapeutic specificity in the clinical use of these agents.

4) To study the mechanisms whereby manipulations of immune reactivity as described above affect host defences against infection, tumor surveillance, and propensity towards auto-immune states.

5) To study the functional capabilities of eosinophils and mononuclear cells in the idiopathic hypereosinophilic syndrome in order to elucidate the mechanisms whereby these cells cause invasion of tissue and subsequent tissue damage in this disease. To use this information to gain a greater understanding of the functional properties of eosinophils in normal states, various diseases and to determine their sensitivity to various therapeutic regimens.

Methods Employed:

1) Much of the methodology is centered around the in vitro culturing of lymphoid cells to determine their varied and numerous functional capabilities. The effects of in vivo chemotherapeutic agents as well as the effects of disease-related altered states of immunity can thus be monitored and explored.

2) Administering corticosteroids to normal volunteers and patients requiring this drug and measuring the specific effects of these agents on the compartmentalization and functional capabilities of monocytes and lymphocyte subpopulations.

3) Labeling a subject's lymphoid cells with ^{51}chromium and reinfusing the cells back into their circulation to determine the effects of disease states and of chemotherapeutic agents on the viability and circulation patterns of these cells.

4) Identifying subpopulations of lymphoid cells by their surface markers and functional capacities, and determining the change in relative proportions of such cells in disease states and during chemotherapy. Identifying specific lymphocyte subpopulations as the cell type involved in lymphocyte-mediated disease in man and in experimentally-induced disorders in animals.

5) Producing altered states of immunity in experimental animals by x-irradiation, surgical removal of lymphoid organs, or administration of immunosuppressive agents in order to determine precisely the requirement of various immunological and non-specific factors involved in host defence against infection and in immunological surveillance.

6) Developing models of experimental disease states in animals such as general hypersensitivity and autoallergic disease, graft and tumor rejection and organ specific hypersensitivity disease in order to study the precise mechanisms of disease activity, the cell types involved, the organ distribution of these cells, and the sensitivity of such disease states to various chemotherapeutic regimens.

7) Performing bone marrow aspirates in normal subjects and patients with various diseases prior to, during, and following therapy. Obtaining purified populations of lymphoid cells from these bone marrow cell suspensions by various separation techniques in order to delineate the immunocompetence of bone marrow lymphoid cells in normal man, as well as the alteration of compartmentalization and function of these cells in untreated and treated disease states.

8) Development of a system of primary in vitro stimulation of human bone marrow derived (B) lymphocytes by polyclonal activation as well as specific antigenic stimulation with subsequent measurement of single cell antibody production by a direct hemolysis-in-gel plaque forming cell assay.

Major Findings and Significance to Biomedical Research and the Program of the Institute:

1) A group of patients with Wegener's granulomatosis (a disease characterized by granulomatous vasculitis involving the respiratory tract and kidneys) are being studied. Untreated, the disease is rapidly fatal, and various chemotherapeutic regimens including corticosteroids have been tried previously, and have failed. We are currently following the largest single group of such patients in the world. Over the past several years we have firmly established a particular chemotherapeutic regimen (chronic low dose cytotoxic therapy, particularly cyclophosphamide) as being highly effective

in producing long term remissions which can now begin to be considered cures (greater than five years of freedom from disease activity). These results have now been confirmed by several centers throughout the world.

Besides serving as an excellent study model of a hypersensitivity disease and the effect of cytotoxic therapy on such a disease state, our experience with this disorder has allowed us to already make, and to continue to make, several important and original observations on the effect of low dose cytotoxic therapy on subpopulations of lymphoid cells in man.

Another important contribution which the study of this disease has allowed us to make is the demonstration of the feasibility of successful renal transplantation with long term followup (greater than two years) in certain inflammatory diseases in which renal failure follows previous kidney damage resulting in a patient who has attained clearcut remission and is otherwise well, but requires long-term hemodialysis.

We have now begun to study patients with a variety of other vasculitides such as polyarteritis nodosa and hypersensitivity vasculitis which have proven refractory to corticosteroid therapy. We have instituted therapy with cytotoxic agents (such as cyclophosphamide) used successfully by us in Wegener's granulomatosis, and have thus far had strikingly successful therapeutic results.

2) A major endeavor of the laboratory is the continuation of the elucidation of the precise mechanisms whereby corticosteroids exert their anti-inflammatory and immunosuppressive effects in various immunologically mediated and inflammatory diseases in man. We have previously described the selective and differential effects of corticosteroid administration on monocyte and lymphocyte subpopulations by a combination of compartmentalization or redistribution and direct effect on functional capabilities. We have extended these studies to demonstrate for the first time in man the differential and specific effects of corticosteroid administration on different subpopulations of cytotoxic effector cells mediating killer cell functions against various tissue targets. Such studies have clearcut clinical relevance in the understanding and therapeutic approach towards a number of inflammatory diseases, autoimmune states, and organ transplant rejection. In addition, they allow a new appreciation of some of the potential mechanisms operating in tumor surveillance via effector cell populations.

3) Studies on the specific immunosuppressive effects of cytotoxic agents in patients with various immunologically-mediated diseases have enabled us to correlate the sensitivity of various lymphoid cell populations to cytotoxic agents with the therapeutic efficacy of these agents in certain disease states particularly those described above in #1. Animal models have been established in order to correlate and extend these studies towards delineating the precise effects of these agents on various leukocyte populations and functional capabilities.

4) We have extended our previous studies in which we had isolated [illegible] described the various subpopulations of lymphoid cells in human bone [illegible] We have now demonstrated in normal human bone marrow distinct subpopulations of lymphoid cells capable of cytotoxic killer cell function. These studies have important implications in understanding the graft-versus-host reactions accompanying many bone marrow transplants in addition to furthering our understanding of the potential and actual immunocompetence of human bone marrow lymphoid cells.

5) We have extended our studies on the effect of experimentally induced viral gastroenteritis on the distribution of lymphoid subpopulations in man by investigating the effect of experimentally induced influenza A infection (a disease of world-wide importance and significant morbidity and mortality) on various lymphocyte functional subpopulations.

6) The newest and perhaps most exciting development in the laboratory over the past year has been the establishment for the first time of a highly reproducible system of primary in vitro stimulation of human B lymphocytes by polyclonal B cell activators or antigens with measurement of single cell antibody production by a direct hemolysis-in-gel plaque forming cell assay. This has been accomplished with human tonsil, bone marrow, spleen, and blood.

For many years, immunologists have been trying unsuccessfully to develop such a model of human B cell activation. The availability of such a system will allow us to probe in depth the complex events associated with B cell activation in man. This will allow us to better diagnose, understand, monitor, and hopefully treat various diseases of over or under-production of antibody.

Proposed Course:

This project will continue along the lines which have been described.

Publications:

1. Balow, J. E., Hurley, D. L., and Fauci, A. S.: Cyclophosphamide suppression of established cell mediated immunity: Quantitative versus qualitative changes in lymphocyte populations. J. Clin. Invest. 56: 65-70, 1975.

2. Chusid, M. J., Parrillo, J. E., and Fauci, A. S.: Chronic granulomatous disease in an adult: Diagnosis in a 27 year old with invasive Mycobacterium fortuitum infection. J.A.M.A. 233: 1295-1296, 1975.

3. Chusid, M. J., Gelfand, J. A., Nutter, C., and Fauci, A. S.: Pulmonary aspergillosis, inhalation of contaminated marihuana smoke, chronic granulomatous disease. Ann. Inter. Med. 82: 682-683, 1975.

4. Dale, D. C., Fauci, A. S., Guerry, D. and Wolff, S. M.: Comparison of agents producing a neutrophilic leukocytosis in man: Hydrocortisone, prednisone, endotoxin and etiocholanolone. J. Clin. Invest. 56: 808-813, 1975.

5. Fauci, A. S.: Human bone marrow lymphocytes. I. Distribution of lymphocyte subpopulations in the bone marrow of normal individuals. J. Clin. Invest. 56: 98-110, 1975.

6. Fauci, A. S. and Dale, D. C.: Monocytopenia after prednisone. New Eng. J. Med. 292: 928, 1975.

7. Fauci, A. S. and Dale, D. C.: The effect of hydrocortisone on the kinetics of normal human lymphocytes. Blood 46: 235-243, 1975.

8. Hurley, D. L. and Fauci, A. S.: Disseminated candidiasis. I. An experimental model in the guinea pig. J. Infect. Dis., 131: 516-521, 1975.

9. Hurley, D. L., Balow, J. E., and Fauci, A. S.: Experimental disseminated candidiasis. II. Glucocorticosteroid administration, susceptibility to infection, and immunity. J. Infect. Dis. 132: 393-398, 1975.

10. Dale, D. C., Hubert, R., and Fauci, A. S.: Eosinophil kinetics in the hypereosinophilic syndrome. J. Lab. Clin. Med. 87: 487-495, 1976.

11. Fauci, A. S., Dale, D. C., and Balow, J. E.: Glucocorticosteroid therapy: Mechanisms of action and clinical considerations. Ann. Intern. Med. 84: 304-315, 1976.

12. Fauci, A. S., Balow, J. E. and Pratt, K. R.: Human bone marrow lymphocytes: Cytotoxic effector cells in the bone marrow of normal individuals. J. Clin. Invest. 57: 826-835, 1976.

13. Fauci, A. S.: Pulmonary vasculitis. In, Kirkpatrick, C. H. and Reynolds, H. Y., (Eds.): Immunologic and Infectious Reactions in the Lung. New York, N. Y., Marcel Dekker, Inc. Vol. 1, p. 243, 1976.

14. Fauci, A. S.: Mechanisms of corticosteroid action on lymphocyte subpopulations. II. Differential effects of in vivo hydrocortisone, prednisone, and dexamethasone on in vitro expression of lymphocyte function. Clin. Exp. Immunol. 24: 54-62, 1976.

15. Fauci, A. S., Johnson, R. E. and Wolff, S. M.: Radiation therapy of midline granuloma. Ann. Intern. Med. 84: 140-147, 1976.

16. Fauci, A. S., Balow, J. E., Brown, R., Chazan, J., Steinman, T., Sahyoun, a. I., Monaco, A. P., and Wolff, S. M.: Successful renal transplantation in Wegener's granulomatosis. Amer. J. Med. 60: 437-440, 1976.

17. Gelfand, J. A., Fauci, A. S., Green, I. and Frank, M. M.: A simple method for the determination of complement receptor-bearing mononuclear cells. J. Immunol. 116: 595-599, 1976.

18. Fauci, A. S., Balow, J. E., and Wolff, S. M.: The effect of cytotoxic therapy on the renal lesion of Wegener's granulomatosis. Proc. VI International Congress of Nephrology. 1975. In press.

19. Fauci, A. S. and Wolff, S. M.: Hepatic granulomata. In, Popper, H. and Schaffner, F., (Eds.): Progress in Liver Disease. New York, N. Y., Grune and Stratton, Vol. V., In press.

20. Fauci, A. S., Balow, J. E. and Pratt, K. R.: Cytotoxic effector cells in the bone marrow of normal humans. In Eijsvoogel, V. P., Roos, D., and Zeylemaker, W. P. (Ed.): Proc. 10th Leukocyte Culture Conference. New York, N. Y., Academic Press. In press.

21. Dinarello, C. A., Chusid, M. J., Fauci, A. S., Gallin, J. I., Dale, D. C., and S. M. Wolff: The effect of prophylactic colchicine therapy on leukocyte function in patients with familial Mediterranean fever. Arthritis Rheum. In press.

22. Hunninghake, G. W. and Fauci, A. S.: Quantitative and qualitative effects of cyclophosphamide administration on circulating polymorphonuclear leukocytes. Immunology. In press.

23. Fauci, A. S., Balow, J. E. and Pratt, K. R.: Cytotoxic effector capacity of bone marrow mononuclear cells. Int. Arch. Allergy Appl. Immunol. In press.

24. Balow, J. E. and Fauci, A. S.: Heparin and lymphocyte kinetics: Use in studies of the mechanisms of corticosteroid-induced lymphocytopenia. J. Retic. Soc. In press.

25. Hunninghake, G. W. and Fauci, A. S.: Divergent effects of cyclophosphamide administration on mononuclear killer cells: Quantitative depletion of cell numbers versus qualitative suppression of functional capabilities. J. Immunol. In press.

26. Fauci, A. S. and Pratt, K. R.: Activation of human B lymphocytes. I. Direct plaque forming cell assay for the measurement of polyclonal activation and antigenic stimulation of human B lymphocytes. J. Exp. Med., In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00056-04 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Pathways of Mediator Release and Mechanism of Tissue Injury in Allergic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any) R. W. Colman (Coagulation Lab., Univ. of Pa. Med. School, Phila., Pa.); J. V. Pierce (LPC NHLI NIH)(Project No. Z01 HI 01944-18 HE); S. I. Katz (LD NCI NIH)(Project No. Z01 CBD 365702 D); R. Rosenberg (Dept. Hematology, Beth Israel Hospital, Boston, Mass.)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4-5/12	3-5/12	1

SUMMARY OF WORK (200 words or less - underline keywords)

The function of activated Hageman factor has been shown to be augmented by high molecular weight (HMW) kininogen as a prerequisite for significant conversion of prekallikrein to kallikrein in plasma. Kallikrein then enzymatically activates Hageman factor and this interaction is facilitated by HMW kininogen. Prekallikrein and HMW kininogen circulate in plasma as a non-covalently linked complex. Activated Hageman factor can be slowly inhibited by antithrombin III, however addition of heparin activates antithrombin and inhibition is then virtually instantaneous.

Histamine was shown to enhance eosinophil migration to other chemotactic agents via the H-1 receptor and at higher doses, inhibits eosinophil migration by stimulating H-2 receptors. The inhibition appears to be secondary to raising the cellular level of cyclic AMP. The chemotactic activity of histamine itself is not inhibited by H-1 or H-2 receptor antagonists and may indicate the presence of a new type of histamine receptor.

1) Determination of the role of prekallikrein and high molecular weight kininogen (HMWK kininogen) in the Hageman factor dependent pathways of coagulation and fibrinolysis. Methods for isolating each component will be developed, and its mechanism of activation determined. The role of plasma inhibitors to control the Hageman factor dependent pathways will be assessed. Radioimmunoassays or radial immunodiffusion assays for assessment of the bradykinin forming pathway in human plasma will be developed focusing attention upon the early steps of the intrinsic coagulation and fibrinolytic pathways.

2) Determine the mechanism by which the Hageman factor dependent plasminogen activator converts plasminogen to plasmin.

3) Study the mechanism by which negatively charged surfaces interact with Hageman factor to expose an active site which can initiate coagulation, fibrinolysis, and kinin formation.

4) Study the interaction of histamine with eosinophils in terms of its role as a chemotactic factor and its ability to modulate the response of the eosinophil to other chemotactic factors. The histamine receptors on the eosinophil will also be characterized.

5) Further investigation of the pathogenesis of cold urticaria, dermographism, cholinergic urticaria, and chronic idiopathic urticaria and angioedema, specifically focusing upon identification of the vasoactive principle which causes the defect, identifying any immunologic mechanism which may play a role, and defining the biochemical pathway which is activated in each disorder. Particular attention is given to any role played by IgE antibody, the human basophil and mast cell, and the release of prostaglandins and kinins in the skin.

Methods Employed:

Purification of plasma proteins is performed utilizing affinity chromatography, ion exchange chromatography on Quarternary aminoethyl (QAE) Sephadex and Sulphopropyl (SP) Sephadex, gel filtration, and preparative elution from gels after isoelectric focusing. These methods have been applied to the isolation of unactivated Hageman factor, Hageman factor prealbumin fragments, pre-PTA (coagulation Factor XI), prekallikrein, plasminogen proactivator, plasminogen and the plasma inhibitors α_1 antitrypsin, α_2 macroglobulin, the inhibitor of the activated first component of complement ($C\bar{1}$ INH), inter α trypsin inhibitor, α_1 chymotrypsin inhibitor, and antithrombin III. Purity is evaluated by alkaline disc gel electrophoresis, SDS gel electrophoresis, and isoelectric focusing.

Eosinophil and mononuclear chemotactic activity is assessed in Boyden chambers using partially purified cells from patients with eosinophilia or partially purified monocytes obtained with Hypaque-Ficoll cushions.

Plasma histamine and serotonin levels are determined by the radioenzyme method and plasma levels of bradykinin by radioimmunoassay.

Major Findings:

1) The mechanism of activation of Hageman factor has been elucidated. Binding of Hageman factor to a surface appears to generate an active site which can then activate prekallikrein, Factor XI, and plasminogen proactivator. High molecular weight kininogen was found to be a cofactor which enhances the ability of surface bound Hageman factor to activate each of its substrates. The enhancement is two to three-fold and is stoichiometric in that the enhancement is directly proportional to the quantity of HMW kininogen added. Maximal enhancement is attained when the reactants are in equimolar ratios. The feedback effect of kallikrein upon Hageman factor activation was found to be dependent upon HMW kininogen in two ways. First HMW kininogen is needed to form sufficient kallikrein for this effect to be evident. Second, the ability of kallikrein to activate Hageman factor enzymatically was found to be markedly enhanced in the presence of HMW kininogen. Thus each protein of the kinin forming pathway was found to provide the mechanism for augmenting the activation and function of Hageman factor upon surfaces and provides the trigger for the initiation of coagulation and fibrinolysis.

2) Prekallikrein and HMW kininogen were found to normally circulate as a complex of molecular weight 260,000 which dissociates when plasma is fractionated by ion exchange chromatography. The molecular weight of prekallikrein in HMW kininogen deficient plasma was only 100,000 and when this plasma was mixed with prekallikrein deficient plasma, a complex at molecular weight 260,000 was again observed. Thus prekallikrein and HMW kininogen not only act sequentially to yield bradykinin and act together to activate Hageman factor but are physically associated in plasma in a non-covalent interaction.

3) The plasma inhibitor antithrombin III was found to inhibit intact activated Hageman factor as well as Hageman factor fragments. Inhibition was gradual over a 20 minute time period. Addition of heparin (10 U/m) activated the antithrombin III so that inhibition of Hageman factor was complete within 20 seconds. Binding of Hageman factor to antithrombin III-heparin was observed upon SDS gel electrophoresis and, as assessed by their respective molecular weights, a 1:1 molar complex was formed.

4) The interaction of the Hageman factor dependent plasminogen activator and plasminogen revealed a mechanism similar to that observed upon activation by urokinase rather than streptokinase. Conversion of plasminogen to plasmin was accompanied by cleavage of the molecule from a single chain structure of molecular weight 94,000 to two disulfide linked chains of 55,000 and 27,500 respectively. Incorporation of P^{32} DFP into the plasmin prior to reduction

and alkylation revealed all of the counts on the 27,500 molecular weight moiety indicating that the light chain contains the active site [illegible] plasmin molecular. No binding of I^{125} plasminogen activator to [illegible] was observed. Thus a stoichiometric complex such as that seen [illegible] was not observed.

5) Histamine was found to enhance and inhibit the responsiveness of eosinophils to other chemotactic agents. At 10^{-6}M histamine enhancement was observed and this could be reversed with H-1 receptor antagonists. At higher doses such as 10^{-5}M, inhibition of chemotaxis was observed and this was reversed with H-2 receptor antagonists. Eosinophil migration was also inhibited by agents such as isoproterenol and aminophylline which raise intracellular cyclic AMP and dibutryl c-AMP itself inhibited eosinophil migration. Histamine doses of 10^{-5} to 10^{-4} molar raised the c-AMP level of eosinophils and had a much less effect upon neutrophils. Thus eosinophils appeared to possess both H-1 and H-2 receptors to histamine. At lower doses the H-1 stimulation predominated and at higher doses the effect of H-2 receptor stimulation predominated. The evidence suggests that the inhibition by high dose histamine may be mediated by raising the intracellular level of c-AMP. The chemotactic activity of histamine itself is not inhibited by H-1 or H-2 receptor antagonists and suggests that eosinophils may contain another type of histamine receptor that has not previously been described.

6) A new method for the evaluation of urticaria has been developed in conjunction with Dr. Michael Beaven (NHLI) and Dr. Steven Katz (NCI). This involves the induction of blisters on urticarial lesions by a combination of negative pressure provided by a suction apparatus attached to a vacuum line and a heating coil. Patients with cold urticaria have previously been shown to release histamine into their venous circulation after placement of their hand in ice water. We now have shown that histamine is released directly into the superficial dermis when an ice cube is placed on their skin and assaying the blister fluid developed by this apparatus right on top of the urticarial lesions. Blisters performed on control patients or non-challenged sites on the same patient had significantly less histamine in them. This method is being used in a variety of types of urticaria in which mediators are not detected in the circulation but are clearly being released into the skin.

7) The IgE of a single patient with IgE mediated cold urticaria has been isolated and its interaction with the skin to yield cold dependent histamine release is being studied.

Significance to Biomedical Research and the Program of the Institute:

The interrelated enzymatic pathways consisting of coagulation, fibrinolysis, and kinin-generation have not been extensively studied in human disease. The role played by these enzymes and their vasoactive and chemotactic byproducts are being evaluated in various types of chronic urticaria and angioedema, cold urticaria, cholinergic urticaria, and urticarial erythema multiforme as one aspect of a larger program being developed in the LCI devoted to the

investigation of the etiology, pathogenesis, and therapy of allergic diseases. Hopefully identification of the various mediators and enzymatic pathways activated in these disorders will lead to an understanding of their pathophysiology and hopefully to new modes of therapy.

The identification of the role of the kinin generating proteins in the activation of Hageman factor has important implications for understanding endotoxic shock. Lipid A of certain endotoxins can activate Hageman factor and initiate coagulation and fibrinolysis, and release the vasoactive peptide bradykinin into the circulation. Along with activation of the complement pathways, this may contribute to the tissue pooling and profound shock that can result.

The discovery that histamine is chemotactic for human eosinophils may help explain the mechanism by which these cells are recruited into the vicinity of allergic reactions. Thus histamine as well as the peptides designated ECF_A together mediate this function. The net effect may depend upon the absolute quantity and ratio of these mediators. Both deactivate the cell and high concentrations of histamine inhibit motility which may aid in keeping stimulated cells at the reaction site. Histamine further appears to both enhance and inhibit eosinophil migration to other chemotactic agents and may play a more general role in modulating the function of the eosinophil.

Proposed Course:

To continue the studies outlined above and develop the ability to evaluate all of the mediator systems in patients with allergic diseases. The mechanism of activation of Hageman factor and its substrates, the inhibition of their activities by natural inhibitors, and the generation of chemotactic factors will be pursued. Assays will be further developed and applied to measure each factor functionally and as a protein in human plasma. The role of IgE, the basophil, and the tissue mast cell in human allergic diseases will be evaluated with the emphasis upon various types of urticarias and angioedemas. The role of histamine as an eosinophil chemotactic factor will be further investigated as it relates to eosinophil function in allergy. Its interaction with ECF_A (the peptides Val Gly Ser Glu and Ala Gly Ser Glu) will be assessed as it relates to the recruitment and function of eosinophils. Methods for the assessment of histamine, serotonin, kinins and prostaglandins within skin and/or blister fluids will be applied to patients with idiopathic urticaria and angioedema, cold urticaria, cholinergic urticaria, and dermographism.

Publications:

1. Burnett, J., Meier, H.L., and Kaplan, A.P.: A study of the mediators present in the hematocyst venoms of the Sea Nettle, Sea Wasp, and Portuguese Man-O-War. Comp. Biochem. Physiol. 51c: 153-156, 1975.

2. Clark, R.A.F., Gallin, J.I., and Kaplan, A.P.: The selective eosinophil chemotactic activity of histamine. J. Exp. Med. 142: 1462-1476, 1975.

[illegible] and role in [illegible]. London, W. B. [illegible].

4. Colman, R.W., Bagdasarian, A., Talamo, R.C., Scott, C.F., Seavey, M., [illegible], Pierce, J.V., and Kaplan, A.P.: Williams trait: Human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman factor dependent pathways. J. Clin. Invest. 56: 1650-1662, 1975.

5. Kaplan, A.P. and Austen, K.F.: Activation and control mechanism of Hageman factor dependent pathways of coagulation, fibrinolysis, and kinin-generation and their contribution to the inflammatory response. J. Allergy and Clin. Immunol. 56: 491-506, 1975.

6. Kaplan, A.P., Gray, L., Shaff, R.E., Horokova, Z., and Beaven, M.A.: In vivo studies of mediator release in cold urticaria and cholinergic urticaria. J. Allergy Clin. Immunol. 55: 422-429, 1975.

7. Kay, A.B. and Kaplan, A.P.: Chemotaxis and hemostasis. Brit. J. Haemat. 31: 1-5, 1975.

8. Miller, W.L., Doppman, J.L., and Kaplan, A.P.: Renal arteriography following anaphylactic reaction to contrast material. J. Allergy and Clin. Immunol. 56: 291-295, 1975.

9. Neva, F.A., Kaplan, A.P., Pacheco, G., Gray, C.L., and Danaraj, T.J.: Tropical eosinophilia - A human model of parasitic immunopathology with observations or serum IgE levels before and after treatment. J. Allergy and Clin. Immunol. 55: 422-429, 1975.

10. Kaplan, A.P.: The pharmacologic modulation of mediator release from human basophils and mast cells: Rationale for the therapy of asthma. In Kirkpatrick, C.H. and Reynolds, H.Y. (Eds.): Immunologic Reactions of the Lung. New York, N.Y., Marcel Dekker, 1976, Vol. 1, pp. 445-459.

11. Colman, R.W., Bagdasarian, A., Talamo, R.C., and Kaplan, A.P.: Williams trait: Combined deficiency of plasma plasminogen proactivator and kininogen. In Pisano, J. (Ed.): The Kallikrein System in Health and Disease. U.S. Government Printing Office, Washington, D.C., Fogarty International Center Proceedings, No. 27. In press.

12. Kaplan, A.P.: The Hageman factor dependent fibrinolytic pathway of human plasma. In Mammen, E.F. (Ed.): Seminars on Thrombosis and Hemostasis. In press.

13. Kaplan, A.P.: Urticaria and angioedema. In Middleton, E., Reed, C., and Ellis, E. (Eds.): Allergy: Principles and Practice. C. V. Mosby. In press.

14. Kaplan, A.P. and Beaven, M.A.: In vivo studies of the pathogenesis of cold urticaria, cholinergic urticaria, and vibration-induced swelling. J. Invest. Derm. In press.

15. Kaplan, A.P., Meier, H.L., and Mandle, R.J., Jr.: The Hageman factor dependent pathways of coagulation, fibrinolysis, and kinin-generation. In Seminars in Thrombosis and Haemostasis. In press.

16. Kaplan, A.P., Meier, H.L., Yecies, L.D., and Heck, L.W.: Hageman factor and its substrates. The role of Factor XI (PTA), prekallikrein, and plasminogen proactivator in coagulation, fibirnolysis, and kinin-generation. In Pisano, J. (Ed.): The Kallikrein System in Health and Disease. U.S. Government Printing Office, Washington, D.C., Fogarty International Center Proceedings, No. 27. In press.

17. Meier, H.L., Scott, C.F., Mandle, R.J., Jr., Webster, M.E., Pierce, J.V., Colman, R.W., and Kaplan, A.P.: Activation of human Hageman factor: Interaction of Hageman factor with high molecular weight kininogen and prekallikrein. Ann. N. Y. Acad. Sci. In press.

18. Metzger, W.J., Kaplan, A.P., Irons, J., and Patterson, R.: Hereditary vibratory angioedema: Confirmation of histamine release in a type of physical hypersensitivity. J. Allergy Clin. Immunol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00057-03 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Basic Studies on Pathogenic Fungi

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K. J. Kwon-Chung Research Microbiologist LCI NIAID

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 - 11/12	2	11/12

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to cover basic and applied aspects of various pathogenic and nonpathogenic fungi including their morphology, pathogenicity, sexual life cycle and genetics. The topics of present interest include: 1) the sexual reproduction cycle of Cryptococcus neoformans; 2) detection of the natural reservoir of serotypes A, B, C, D; 3) genetics of serotypes and mating types; 4) virulence among two mating types of 4 serotypes.

Objectives:

Objectives of the project cover both basic and applied aspects of various pathogenic fungi, including their morphology taxonomy, pathogenicity, life cycle and genetics.

Methods Employed:

More than a hundred isolates of Cryptococcus neoformans, both clinical and natural origin, have been cultured singly or in combination on malt extract agar and incubated at the various temperatures for 2 to 3 weeks. Observations on the morphogenesis of the sexual state were made by both light and electron microscopy. Cytological staining was performed using basic fuchsin solution. Isolation of sexual spores was done by micromanipulation.

Major Findings:

The sexual state of Cryptococcus neoformans was first found among the strains of serotypes A and D. When two compatible strains, α and a, are mixed on malt extract agar and incubated at the temperatures between 15 to 37°C, yeast cells of two opposite mating types conjugate and produce long filament with clamp-connections. Basic fuchsin staining showed that these hyphae are dikaryotic and nucleus migrate through the clamp-connections to establish the dikaryotic state. Long slender basidia are formed singly or in groups on the side or at the tip of the hyphae. Basidiospores are produced on the apex of the basidia by repeated budding typically from four spots. Cytological study revealed that diploidization and meiosis occur in basidia. Basidiospores are usually uninucleate and subglobase to lemon shaped, ranging from 1.2 to 1.8 µm. Unlike the asexual yeast cells, the sexual spores are noncapsulated. The sexual spores germinate into yeast cells of either self-sterile α or a. Genetic analysis using two markers indicated true meiosis and bipolar sexuality. The perfect state of A, D serotype was designated as Filobasidiella neoformans. Strains of B, C serotype required biotin-sucrose medium for the completion of sexual life cycle. The morphogenesis leading to the sexual state of B, C serotypes is generally the same as F. neoformans but produces long slender bacillary form spores (3 to 8 µm x 1 to 1.5 µm). The second species is designated as F. bacillispora. The interspecific cross between F. neoformans and F. bacillispora does not occur on malt agar and sometimes occurs on biotin-sucrose medium. Basidiospores produced in such crosses are usually nonviable.

Electron microscopic study revealed typical basidiomycetous dolipores with no parenthesomes.

Significance to Biomedical Research and the Program of the Institute:

Finding of sexual states Cryptococcus neoformans provides an unambiguous criterion to establish a phylogenetic relationship between this pathogen and other fungi.

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[illegible]:

[illegible] major effort will be directed to: 1) investigate the physiological differences between Filobasidiella neoformans and F. bacillispora; 2) compare the virulence between α and a type within and between the two species: 3) investigate nuclear state and ploidy of self-fertile isolates in the two species; 4) study frequency of the two mating types among natural and clinical isolates.

Publications:

1. Kim, S. J., Kwon-Chung, J. J., Milne, G. W. A., and Prescott, B.: Polyene resistant mutants of Aspergillus fennelliae: Identification of sterols. Antimicrob. Agents Chemother. 6: 405-410, 1974.

2. Kim, S. J. and Kwon-Chung, K. J.: Polyene-resistant mutants of Aspergillus fennelliae: Sterol content and genetics. Antimicrob. Agents Chemother.6: 102-113, 1974.

3. Kwon-Chung, K. J.: A new pathogenic species of Aspergillus in Aspergillus fumigatus series. Mycologia 67: 770-779, 1975.

4. Kwon-Chung, K. J., Schwartz, J. S., and Rybak, B.: Pulmonary fungus ball caused by Cladosporium cladosporidioides. Am. J. Clin. Path. 64: 564-568, 1975.

5. Kwon-Chung, K. J.: Perfect state (Emmonsiella capsulata) of the fungus causing large-form African histoplasmosis. Mycologia 67: 980-990, 1975.

6. Kwon-Chung, K. J., Young, R., and Orlando M.: Pulmonary mucormycosis caused by Cunninghamella elegans. Am. J. Clin. Path. 64: 545-548, 1975.

7. Kwon-Chung, K. J.: A new genus Filobasidiella, the perfect state of Cryptococcus neoformans. Mycologia 67: 1197-1200, 1975.

8. Kwon-Chung, K. J. and Popkin, T. J.: Ultrastructure of septal complex in Filobasidiella neoformans (Cryptococcus neoformans) J. Bacteriol. 126: 524-528, 1976.

9. Meyers, W. M., Dooley, J. R., and Kwon-Chung, K. J.: Mycotic granuloma caused by Phialophora repens. Am. J. Clin. Path. 64: 550-555, 1975.

10. Emmons, C. W., Binford, C. H., Utz, J. P. and Kwon-Chung, K. J.: Medical Mycology. Philadelphia, Lea and Febiger. 550 pp. In press.

11. Kwon-Chung, K. J.: Morphogenesis of Filobasidiella neoformans, the sexual state of Cryptococcus neoformans. Mycologia 68: In press.

12. Kwon-Chung, K. J.: A new species of Filobasidiella, the sexual state of Cryptococcus neoforms. B and C serotypes. Mycologia, In press.

13. Wong, V. G., Kwon-Chung, K. J., and Hill, W. B.: Koch's postulates and experimental ocular histoplasmosis. International Opthal. Clin. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00058-03 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Pathogenesis and Chemotherapy of Herpes Virus Infections in Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. Mazur	Clinical Associate	LCI NIAID
	R. Reichman	Clinical Associate	LCI NIAID

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 - 8/12	1 - 8/12	1

SUMMARY OF WORK (200 words or less - underline keywords)

The pathogenesis of Herpesvirus infections in man and in experimental animals are investigated by characterization of viral isolates, and by examination of immunological response with particular emphasis on cell-mediated immunity. Patients with recurrent herpes simplex infections have elevated levels of serum antibody, and normal or elevated levels of lymphocyte cytotoxicity against virus infected target cells. The efficacy and mechanism of action of antiviral compounds are examined in clinical trials, as well as in vitro. One such antiviral compound, adenine arabinoside (ara-A) appears to have efficacy in the treatment of herpes-zoster in the immunosuppressed, with little toxicity. Additional trials in Herpes Simplex infections in the immunosuppressed are underway, and trials in other Herpesvirus infections are planned for the next year. In vitro data indicates that ara-A may selectively inhibit viral directed DNA synthesis.

PHS-6040

Objectives:

1) To determine the pathogenesis,host defense mechanisms, and natural history of herpes virus infections in man, in both host-defense compromised and intact hosts.

2) To evaluate the efficacy of antiviral agents in the treatment of herpes virus infections in man. These infections include herpes simplex, herpes zoster, cytomegalovirus and EB virus.

3) To develop in vitro methods to study the activity, mechanisms of actions, pharmacology, and interrelationships of antiviral agents.

4) To develop laboratory animal models in which to study pathogenesis of herpes virus infections and the effect of antiviral substances.

Methods Employed:

1) Patients with infections with Herpes Simplex, Herpes Zoster, Cytomegalovirus, and Epstein-Barr Virus are referred to the Medical Virology Section from the Clinical Center, National Naval Medical Center, or other local hospitals or practicing physicians. Patients who are hospitalized are studied at the Clinical Center or at NNMC, while those who are ambulatory are seen in the out-patient clinic of the LCI.

2) Viruses isolated from the above patients are characterized in human diploid fibroblasts and in human fetal tracheal and intestinal organ cultures. The effect of antiviral compounds, such as adeninine arabinoside, cytosine arabinoside, idoxuridine, and phosphonoacetic acid on clinical isolates are studied in tissue and organ culture.

3) Host defense responses to herpetic viral infections are examined in the above patients. These include: (a) levels of circulating and locally produced neutralizing and complement fixing antibodies; (b) cell-mediated immune responses, i.e., skin test sensitivity, mitogen and antigen stimulation of peripheral lymphocyte cultures, lymphocyte cytotoxicity against virus-infected target cells, and circulating B to T cell proportions; and (c) systemic and local (e.g., vesicle) interferon production.

4) The clinical efficacy of ARA-A in the treatment of herpes zoster and herpes simplex infections in the immunosuppressed is investigated in a randomized, controlled, double-blind study in collaboration with a NIAID-supported nationwide trial of which the principal investigator is project officer.

Major Findings:

1) Recurrent Herpes-simplex infections of the oro-facial and genital areas in man.

a. An out-patient program was established to study patients with these infections, with the subsequent enrollment of 61 patients. The clinical characteristics of recurrent infections, including provoking factors, sites and duration of lesions, and accompanying symptomalogy were compiled.

b. Virology: All recurrent oro-facial infections were type 1, while 87% of genital infections were type 2 and 13% were type 1. Comparison of *in vitro* growth characteristics failed to differentiate viruses found in recurrent infections from those in primary infections.

c. Immunological responses: Recurrent infections occurred in the presence of high levels of circulating neutralizing antibodies against the homologous virus type: (Mean geometric titer: 91 (HSV-1) and, 93 (HSV-2). A reproducible assay of direct lymphocytotoxicity against tissue culture cells (51 CR labeled human prostate adenoma cells) acutely infected with the homologous virus type was established. Individuals with recurrences had equal or greater levels of cytotoxicity than did matched, sero-positive controls without recurrences. When acute lesions were present, percent cytotoxicity (30 ± 6) was significantly elevated above that seen when lesions were absent (9.5 ± 2.3) in HSV-1 infections, as well as in HSV-2 infections (23 ± 4 vs 6.1 ± 1.4). Similar studies were carried out employing chronically, rather than acutely infected target cells. With these cells, cytotoxicity was 6 to 9% in all groups studied, and did not rise when acute lesions were present. These findings suggest that different antigens may be expressed in chronically vs acutely infected target cells, and that cytotoxic responses to these antigens may reflect the activity of infection.

The effector cell in this system, as determined by mononuclear cell fractionation, appears to be a non-phagocytic, non-T lymphocyte.

2) Clinical trials of systemically administered ara-A in Herpes Simplex and Zoster infections in the immunosuppressed.

a. The past year witnessed the conclusion of the crossover study of herpes zoster with subsequent analysis of 77 patients from 22 institutions, including 11 from this unit. Analysis of this data demonstrated that patients treated with ara-A manifested statistically significant reductions in new lesion formation, virus excretion, and acute pain. Because the effect on post herpetic sequelae and on visceral dissemination could not be assessed, a non-crossover study was initiated, and 7 patients have been enrolled from this unit in that study.

b. Five immunosuppressed patients with progressive mucocutaneous infections were entered in the ara-A trial. Virus excretion appeared to be reduced during administration of the compound, but the clinical course of infection could not be analyzed because of the small number of patients.

c. Disseminated CMV infections in three bone-marrow transplant recepients were treated with 10 mg/kg of ara-A IV for 5 days, with no effect on virus shedding or on progression of pulmonary lesions. Two of 3 patients who died had widespread CMV present at autopsy.

3) Effect of Ara-A on Epstein Barr Virus (EBV)

We previously reported that Ara-A inhibited EBV in a producer lymphoid line, P3HR-1. The mechanism of inhibition was further investigated by selective analysis of EBV antigens by immunofluorescence. Ara-A selectively inhibits the expression of a structural viral capsid protein (VCA), but does not inhibit the non-structural protein, EA. In the non-producer Raji line, Ara-A does not induce EA, nor does it prevent superinfection. This mechanism of action is consistent with a selective inhibition of viral directed DNA synthesis. In contrast, IUDR and Ara-C were found to induce EA, as well as to inhibit host cell replication. These properties may account for the relative toxicity of both ara-C and IUDR as compared to Ara-A, in clinical trials in man.

4) The previously described guinea pig models for systemic herpes-simplex virus infections were employed to evaluate in vivo efficacy of ara-A, PPA and an interferon inducer, Poly ICLC. Pre-treatment of adult, sero-negative guinea pigs with Ara-A and PPA at >50 mg/kg reduced the magnitude of infection and viremia in intact animals, but had little effect on infections in immunocompromised animals. Topically administered PICLC had no effect on cutaneous lesions. Additional models for genital infections were established in guinea pigs with intravaginal inoculation of HSV-1 and HSV-2, as well as in pregnant guinea pigs for study of neo-natal HSV infections.

Significance to Biomedical Research and the Program of the Institute:

Herpes virus infections are a significant and widespread cause of morbidity in normal adults and children, as well as in neonates and in the immunosuppressed. Herpes simplex encephalitis is the most common fatal encephalitis in the United States. Herpes genitalis appears to be increasing in incidence in the United States and has been associated with cervical cancer. There is currently no treatment of established efficacy for these infections. Studies on the pathogenesis and host response in these infections should further define their risk to man, as well as suggest control measures. The availability of compounds such as ARA-A and PPA, which possess anti-herpes activity in vitro and which are relatively nontoxic, offer a promising modality for the treatment of these infections. Studies with these agents can serve as models for the evaluation and use of antiviral drugs which may be subsequently developed.

Proposed Course:

Clinical trials with anti-viral agents will continue and be expanded, including the initiation of a trial of topical therapy in the Herpes Simplex

genitalis population described above. The effect of ara-A on EBV in vivo in man will be studied by examining viral excretion, antibody response, and clinical parameters in immunosuppressed patients who receive the compound. The molecular mechanism of action of ara-A will also be investigated. Host response to herpes virus infections in patients will continue to be studied, with continued emphasis on cell mediated immunity and its relationship to latency and reactivation. Antigens which elicit cell mediated immune responses will be characterized. The guinea pig model will also be employed to examine pathogenesis and to evaluate promising anti-virals.

Publications:

1. Dolin, R. and Smith, H. S.: Antiviral activity of adenine arabinoside and I doxuridine in human fetal intestinal and tracheal organ cultures. J. Infect. Dis. 132: 287-295, 1975.

2. Dolin, R., Gill, F. A., and Nahmias, A. J. Primary Herpesvirus Hominis type 1 infection in man: Evidence for venereal spread. Am. J. Venereal Dis. 2: 13-16, 1975.

3. Levin, M. J., Oxman, M. N., Alford, C. A., Whitley, R., Ch'ien, L. T., Galasso, G., Dolin, R., et al. (Boston Interhospital Virus Study Group and the NIAID-Sponsored Cooperative Antiviral Clinical Study): Failure of high dose 5-iodo-2-deoxyuridine in the therapy of herpes simplex virus encephalitis. Evidence of unacceptable toxicity. New Engl. J. Med. 292: 600-603, 1975.

4. Ch'ien, L. T., Whitley, R. J., Alford, C. A., Galasso, G. J., Dolin, R., et al. NIAID Collaborative antiviral study: Preliminary results with Ara-A therapy of herpes zoster in the immunosuppressed. J. Infect. Dis. In press.

5. Coker-Vann, M., and Dolin, R.: Effect of adeninine arabinoside on Epstein-Barr Virus in vitro. J. Infect. Dis. In press.

6. Whitley, R. J., Ch'ien, L. T., Dolin, R., Galasso, G. J., and Alford, C.A., (Eds.) NIAID Collaborative Antiviral Study. Adeninine Arabinoside Therapy of Herpes Zoster in the Immunosuppressed. N. Engl. J. Med. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00059-03 LCI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Biology of Human and Animal Parvoviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. Dolin Head, Medical Virology Section LCI NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated as a separate project. Work under this project for 1975-76 is reported under project #Z01 AI 00054-04.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00154-01 LCI

PERIOD COVERED

September 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunologic, Neurophysiologic, Biochemical and Cellular Events in Immediate Hypersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D. D. Metcalfe	Clinical Associate	LCI NIAID
	W. R. Henderson	Clinical Associate	LCI NIAID
	J. I. Gallin	Senior Investigator	LCI NIAID
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COOPERATING UNITS (if any)

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SUMMARY OF WORK (200 words or less - underline keywords)

The biochemical events governing the immunologically-induced secretion of the mediators of anaphylaxis from human lung tissue and rat mast cells are being studied. Both Acetylcholine and PGF_{2a} enhance mediator release while inducing massive increases in cyclic GMP in lung tissue suggesting a role for cyclic GMP levels as a modulator of mediator release. Colchicine suppresses mediator release from human lung tissue and rat mast cells but only after disaggregation of microtubules by cold suggesting that microtubules may be involved in mediator release. And finally, a potentially important role for the eosinophil in parasitic diseases has been delineated in schistosomiasis and is now being studied.

Project Description:

Objectives:

The goals of my laboratory are to expand our knowledge of all aspects of immediate hypersensitivity reactions. Our particular expertise and focus is on the biochemical events which occur concomitant to the triggering of allergic reactions and the neurophysiologic mechanisms which potentially modulate these events.

We are concentrating on four primary areas: 1) Employing human lung tissue, we study "asthma in a test tube" in order to understand the immunologic triggers, biochemical concomitants and neurophysiologic controls of mediator release as well as a search for new effector molecules; 2) Rat peritoneal mast cells as a pure source of target cells which may be readily studied in vitro and may amplify as well as open new areas of interest in relationship to the human lung model; 3) Clinical allergic asthma in an extensive protocol designed to expand our understanding of the neurophysiologic mileau of allergic individuals; and 4) The mechanism of eosinophil enzyme secretion in parasitic diseases as well as the possible role of the eosinophil in allergic reactions.

Methods Employed:

1) Human lung model: Lungs removed, usually for cancer resection, almost always contain large amounts of normal, functioning lung tissue. We obtain these lungs in cooperation with the surgical and pathology departments of all the major hospitals in the Washington and Maryland areas. The lung tissue is washed, fragmented and replicated into 200 mg "chunks" and subsequently incubated in dilutions of allergic serum for 2 hours at 37°C or 18 hours at 25°C. After this "sensitization" period, the serum is removed and the fragments challenged with allergin. The interaction of the allergin with tissue (mast cell) bound IgE induces the release of histamine, slow reacting substance of anaphylaxis (SRS-A), eosinophil chemotaxic factor of anaphylaxis (ECF-A), prostaglandins E_1 and F_{2a} (PGE_1 and PGF_{2a}) and platelet activating factor (PAF). These mediators may be assayed on the isolated terminal portion of the guinea pig's ileum (Histamine, SRS-A), chemotactic chambers (ECF-A), radioimmunoassay (PGE_1 and PGF_{2a}) and ^{14}C-serotonin release from rabbit or human platelets (PAF).

We have been determining alterations in the tissue levels of cyclic AMP and cyclic GMP after treating lung with various agonists in order to modulate the release of mediators. Cyclic AMP may be measured by either a protein kinase binding assay or radioimmunoassay while cyclic GMP is determined by radioimmunoassay after acetylation.

2) Rat mast cells: Rat peritoneal and pleural mast cells are obtained by lavage of these cavities in freshly sacrificed male Sprague-Dawley or ACI rats. The yield of cells contains 5% mast cells or approximately 500,000 to

1 million/animal. The cells are used either in mixed cell suspension or after purification by spinning them into Ficoll cushions.

3) Clinical asthma study: The methods employed in this study are covered in detail in the clinical protocol and include: 1) measurement of cutaneous blood flow by xenon^{133} disappearance, 2) pupilary responses as measured by a binocular pupillometer, 3) airway obstruction as measured by flow-volume curves with and without helium inhalation, 4) serum cyclic nucleotide responses after beta adrenergic stimulation as measured by the above detailed methods, and 5) eccrine sweat responses as measured after intracutaneous Mecholyl administration.

4) Eosinophils: Human eosinophils obtained from patients with idiopathic hypereosinophilia or from allergic patients with high grade eosinophilia or from schistosomiasis patients react with schistosoma egg antigen on sepharose 4B beads in the presence of specific antigen with the release of eosinophil-specific enzymes such as aryl sulfatase. This release reaction is antigen and antibody specific and is markedly enhanced by complement. Aryl sulfatase is measured by the conversion of paranitrocatechol to paranitrocatechol sulfate. Other enzymes which are usually assayed include beta glucuronidase, lactic dehydrogenase and lysozyme; all by standard methods.

Major Findings:

Having only arrived at the NIH last fall, the major portion of my time was spent in establishing this laboratory. Over the past few months, the laboratory has been functioning in its research capacity and our findings include:

A) Cyclic nucleotide levels in human lung are 109 picomoles cyclic AMP/mg protein and 15.2 picomoles cyclic GMP/mg protein in 12 lungs. Acetylcholine and PGF_{2a} both induce immediate increases in cyclic GMP without effecting cyclic AMP levels. These alterations in cyclic nucleotide levels are both kinetically- and dose-related to marked enhancement of the antigen-induced release of histamine and SRS-A. Suppression of prostaglandin synthesis with 10 ug/ml acetylsalicylic acid has no effect on mediator release or nucleotide levels, either baseline or after acetylcholine or PGF_{2a} stimulation.

B) Inhibition of microtubular aggregation by colchicine was found to be an ineffective inhibitor of both rat and human mast cell release under standard conditions. Indeed, the only method of suppression with colchicine was found to be incubation at 4°C in order to facilitate disaggregation of the microtubules so that reaggregation was suppressable with colchicine.

In contrast, the microtubular stabilizing agent, heavy water, was found to be a mild to moderate inhibitor of mediator release.

C) The human eosinophil was found to be the rather exclusive repository of arylsulfatase, to have slightly more beta-glucuronidase the neutrophils and

to have much less lysozyme. The release of these enzymes appears to require a potent phagocytic stimulant as interaction with histamine, SRS-A and PGF_{2a} failed to initiate release. Specific antigen afixed to Sepharose 4B plus antibody directed against the antigen will initiate release and this reaction is markedly augmented by complement activation.

D) The clinical asthma study has not been initiated at the time of this writing.

Significance to Biomedical Research and the Program of the Institute:

Increased understanding of the mechanisms of immediate hypersensitivity and its controls may eventually make it possible to better treat the 44 million Americans with allergic rhinitis or the 15 million with asthma. The model systems described herein have been employed to test potential allergy-preventing drugs and their mechanism of action. The immediate significance of this research may best be realized by the understanding that the two newest agents for treatment of asthma (cromalin sodium and Sch-1000, an atropine-like agent) are offshoots of studies involving these models. The long range signficance relates to the mast cell as a single cell endocrine organ in which to study secretory mechanisms and controls.

While the studies employing human lung tissue are directly applicable to asthma, they are hampered by the mixed cellular contents of lung. By contrast, both the rat peritoneal mast cell and human eosinophil may be studied in a homogeneous cell population and are therefore amenable to very sophisticated analysis.

Findings from the above studies are being directly translated into clinical protocols involving asthma and urticaria. This unique combination of basic science and clinical study reflects the goals of the NIH, and the LCI in particular.

Proposed Course:

We plan to continue the type of studies outlined herein with amplification on certain areas. We are initiating an analysis of the role heparin plays in allergic phenomena employing both of our *in vitro* models and also plan to study mucus secretion from lung tissue.

Collaborative efforts are now being established to study mast cell secretion with freeze fracture techniques with Dr. Pinto Di Silva and with an, as yet, unnamed transmission electron microscopist.

As our interests closely approximate those of Dr. John Gallin of the LCI studying human polymorphonuclear leukocytes, we are initiating a collaborative effort to study the initial events in chemotaxis.

Publications:

1. Garovoy, M.R., Strom, T.B., Kaliner, M., and Carpenter, C.B.: Antibody-dependent lymphocyte mediated cytotoxicity (Ab-LMC). Mechanism and modulation by cyclic nucleotides. Cell. Immunol. 20: 197-205, 1976.

2. Kaliner, M.: The cholinergic nervous system and immediate hypersensitivity. I. Eccrine sweat responses in allergic patients. J. Allergy Clin. Immunol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00155-01 LCI

PERIOD COVERED

July 1, 1975 to April 30, 1976

TITLE OF PROJECT (80 characters or less)

Leukocyte Chemotaxis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R. A. F. Clark	Clinical Associate	LCI NIAID
	M. S. Klempner	Clinical Associate	LCI NIAID
	C. H. Kirkpatrick	Head, Clinical Allergy and Hypersensitivity Section	LCI NIAID
	J. A. Kazmierowski	Clinical Associate	LCI NIAID
	A. P. Kaplan	Head, Allergic Diseases Section	LCI NIAID
	H. Y. Reynolds	Head, Bacterial Diseases Section	LCI NIAID

*Cooperating Units (con't): Z01 HL 00607-03 CM); E. F. Neufeld (ARB NIAMDD NIH); J. C. Kauffmann (POB NCI NIH)

COOPERATING UNITS (if any) D. W. Alling (OSD NIAID NIH); H. Malech (LCBGY NCI NIH); E. K. Gallin (Neurobiology Dept., Armed Forces Radiobiology Research Institute, National Naval Medical Center, Bethesda, Md.); J. A. Sandler (IR LCM NHLI NIH); V. A. Manganiello (IR LCM NHLI NIH); M. Vaughn (IR LCM NHLI NIH)(Project No.*

LAB/BRANCH

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SECTION

Clinical Physiology Section

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NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	--

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations of the mechanism of leukocyte interaction with chemotactic factors have included demonstration of the requirement of calcium and magnesium for leukocyte migration. Shifts in intracellular calcium by the calcium ionophore A23187 suggested calcium is important in modulating cyclic nucleotide metabolism.

Levamisole increased neutrophil and monocyte in association with increases in cGMP. Four patients with defective leukocyte chemotaxis, high IgE and recurrent pyogenic infections were given levamisole and in each case significant improvement of in vitro chemotaxis was demonstrated after in vivo levamisole.

In related studies specific orientation of neutrophil centrioles, microtubules and submembranous microfilaments was demonstrated during chemotaxis. Migration requires microfilaments whereas direction appears dependent on microtubule assembly. In addition PMN granules orient in the leading front of migrating PMNs. In related studies a preferential release of specific granules during migration in vitro and exudation in vivo was demonstrated. The mechanisms of modulating the recruitment of leukocytes at inflammatory-immune sites is being actively studied in leukocytes obtained from normal subjects and patients with defective leukocyte migration.

Project Description:

Objectives:

1) To study the role of divalent cations in modulating leukocyte locomotion and the mobilization and release of granular enzymes.

2) Evaluate the role of the cytoskeleton (microtubules and microfilaments) in leukocyte migration.

3) Study the mobilization and release of polymorphonuclear leukocyte granules during migration in vitro and during exudation in vivo.

4) Study the effects of various granule enzymes on modulating the inflammatory response.

5) Study the effects of levamisole on the chemotactic responsiveness of leukocytes obtained from normal subjects and patients with disorders of chemotaxis.

6) Evaluate phagocytic cell function in patients with recurrent pyogenic infections and identify patients with defective leukocyte migration.

7) Evaluate the chemotaxis of eosinophils with particular emphasis on histamine as a selective chemoattractant for eosinophils.

8) Develop sophisticated yet simple approaches for quantitating leukocyte migration.

Methods Employed:

Peripheral blood leukocytes were separated from heparinized whole blood by dextran sedimentation or hypaque-ficoll separation. Chemotaxis was evaluated using a radioassay developed in this laboratory using ^{51}Cr labeled leukocytes and a double micropore filter Boyden chamber. For certain experiments a morphologic assay was used employing a single micropore filter and measuring the distance migrated into the filter by the population of responding cells.

Calcium flux studies were performed by measuring the release of ^{45}Ca from prelabeled cells (efflux) or the uptake of ^{45}Ca by leukocytes (influx).

The surface charge of human leukocytes was measured using a Zeiss cytopherometer. The electrophysiology of the interaction of chemotactic factors with cultivated human macrophages was measured using standard intracellular recording techniques. Leukocyte cyclic nucleotide levels were determined with standard radioimmune assay techniques.

The cytoskeleton of polymorphonuclear leukocytes was studied during conditions of chemotaxis using standard chemotactic chambers and very small

(0.45 μm) micropore filters. These small filters impeded leukocyte migration but permitted pseudopod penetration and established a fixed orientation of leukocytes in a gradient of chemotactic stimulus. After fixation serial sections of adjacent cells were studied using standard electron microscopy techniques.

Polymorphonuclear leukocyte granules were separated and fractionated using sucrose-gradient techniques and various enzyme markers were utilized to identify granule types.

Major Findings:

1) Extracellular calcium is required for leukocyte chemotaxis. During interaction of leukocytes with chemotactic factors there is a net inhibition of uptake of extracellular calcium by leukocytes and enhancement of release of calcium probably reflecting shifts in the distribution of intracellular calcium.

2) Agents which cause a net uptake of calcium inhibit leukocyte chemotaxis.

3) Pharmacologic elevation of intracellular cAMP inhibits chemotaxis and elevation of intracellular cGMP amplifies chemotaxis (Manganiello).

4) Serotonin and ascorbic acid, two agents which amplify leukocyte chemotaxis, increase intracellular cGMP (Sandler).

5) Under conditions of chemotaxis human polymorphonuclear leukocytes have orientation of their nuclei, centrioles microtubules and microfilaments. Studies with colchicine and cytochalasin B suggest microfilaments provide the machinery of locomotion while the centriole and associated microtubule array provide the vector of locomotion (Malech).

6) Incubation of leukocytes with chemotactic factors resulted in a decrease in the net negative surface charge of the cell. This effect of chemotactic factors on leukocyte surface charge was blocked with hydrocortisone sodium succinate but was not affected by colchicine or cytochalasin B.

7) Leukocytes from a patient with severe recurrent pyogenic infections and defective chemotaxis have been shown to have markedly elevated cGMP and tremendous increased assembly of microtubules and microfilaments. Data from this patient suggest a close association between cyclic nucleotide metabolism and regulation of cytoskeleton assembly (Kirkpatrick and Malech).

8) Levamisole (10^{-7}M) amplified normal leukocyte chemotaxis and increased cGMP *in vitro*. *In vitro* levamisole significantly improved the defective chemotactic responses of leukocytes from patients with recurrent infections and elevated IgE. *In vivo* levamisole corrected towards normal the *in vitro* chemotaxis of leukocytes from these patients (Wright and Kirkpatrick).

9) Electrophysiological studies of cultivated human macrophages demonstrated that exposure of these cells to the chemotactic factors in E. coli endotoxin activated sera resulted in a rapid large increase in membrane conductance associated with a membrane hyperpolarization. These electrical events were followed within a few seconds by cell contraction and then pseudopod formation. Control sera and control endotoxin did not produce these effects (E. Gallin).

10) Polymorphonuclear leukocyte (PMN) specific granules are selectively mobilized and released by the pharmacologic stimuli calcium ionophere A23187 and phorbolmyristate acetate and the physical stimuli of adherance to nylon wool. A similar selective release of specific granules was noted during exudation into blisters made by heat and suction in normal subjects or neutrophils obtained from abscesses from a variety of patients (Wright).

11) Specific granules contain an enzyme which activates the complement system to generate the chemotactic factor C5a. Azurophil granules, which are released from cells late after phagocytosis, contain an enzyme which inactivates the chemotactic activity of C5a (Wright).

12) Functional studies of leukocytes collected by continuous flow filtration leukopheresis demonstrated these cells have deficient chemotaxis, phagocytosis and bactericidal activity. Shortened collection times and administration of dexamethasone to donors diminished these functional defects (Wright and Kauffman).

13) Histamine (10^{-6}M) was shown to be a selective chemoattractant for eosinophils. Higher concentrations inhibited chemotaxis and increased eosinophil cAMP. Agents such as metiamide, an H-2 receptor antagonist, prevented the inhibition of chemotaxis and increases in cAMP noted with high histamine concentrations. Several eosinophil receptors appear important in modulating eosinophil responses to histamine (Clark, Sandler and Kaplan).

14) A mathematical analysis of human neutrophil migration rates through cellulose nitrate filters was determined by estimating the diffusion constant (D) of the ordinary random walk formula. The log number of cells at time t present at a distance x μm from the top of the filter was expected to be $(c - x^2)/4Dt$ where c is a constant and D is the diffusion constant. Various patients and normals were studied and the data were compared with a ^{51}Cr-radioassay previously developed in this laboratory. The data demonstrated that the diffusion constant, D, is an accurate way to express the magnitude of migration for an entire population of migrating leukocytes (Clark and Alling).

15) Serial bronchoscopy's of monkeys resulted in the recovery of a chemoattractant which preferentially attracted polymorphonuclear leukocytes. Sequential studies demonstrated the appearance of this material just prior to PMN accumulation in the bronchial washings. In vitro studies demonstrated that alveolar macrophages, when activated with a phagocytic stimulus, release a similar material which, on the basis of G-75 sephadex chromatography, has a

molecular weight less than 5000 daltons. This chemotactic factor is present, preformed in alveolar macrophages but is also synthesized upon stimulation by phagocytosis; cyclohexamide added after phagocytosis blocks subsequent synthesis and elaboration of this chemoattractant by alveolar macrophages (Kazmierowski and Reynolds).

16) Defective leukocyte chemotaxis has been demonstrated in a patient with recurrent pyogenic infections and α-mannosidase deficiency. This patient's PMNs killed bacteria normally. The impaired leukocyte chemotaxis in patients with α-mannosidase deficiency may be the pathologic basis for the recurrent infections seen in this disease.

Significance to Biomedical Research and the Program of the Institute:

The accumulation of leukocytes at inflammatory, immune and allergic sites is recognized as critical for appropriate responses. An understanding of the physiological events regulating cell movement will perhaps provide a basis for pharmacologic manipulation. The observation that levamisole raises intracellular cGMP and corrects the chemotaxis of a select group of patients is exciting in this regard. The morphologic analysis of normal cell migration provides a structural basis for future classification of cellular defects of chemotaxis; analysis of electrophysiological events associated with the triggering of cell movement may be useful in this regard. The control systems for the chemotactic process is also emerging as critical for modulating inflammatory-immune processes and accordingly, the demonstration of sequential release of functionally different classes of PMN leukocyte granules during exudation and phagocytosis should provide a clearer basis for how these processes are modulated and may provide new insights as to the pathophysiology of certain infectious, inflammatory and immune diseases.

Proposed Course:

It is planned to continue some of the studies outlined above.

Publications:

1. Gallin, J.I. and Kirkpatrick, C.H.: Chemotactic activity in dialyzable transfer factor. Proc. Natl. Acad. Sci., USA 71: 498-502, 1974.

2. Gallin, J.I., Durocher, J.R., and Kaplan, A.P.: Interaction of leukocyte chemotactic factors with the cell surface. I. Chemotactic factor-induced changes in human granulocyte surface charge. J. Clin. Invest. 55: 967-974, 1975.

3. Gallin, J.I., Klimerman, J.A., Padgett, G.A., and Wolff, S.M.: Defective mononuclear leukocyte chemotaxis in the Chediak-Higashi syndrome of humans, mink, and cattle. Blood 45: 863-870, 1975.

4. Gallin, J.I. and Wolff, S.M.: Leucocyte chemotaxis: Physiological considerations and abnormalities. Clinics in Haematology 4: 567-607, 1975.

5. Sandler, J.A., Gallin, J.I., and Vaughan, M.: Effects of serotonin, carbamylcholine, and ascorbic acid on leukocyte cyclic GMP and chemotaxis. J. Cell Biol. 67: 480-484, 1975.

6. Wright, D.G. and Gallin, J.I.: Modulation of the inflammatory response by products released from human polymorphonuclear leukocytes during phagocytosis. Inflammation 1: 23-29, 1975.

7. Wright, D.G., Kauffmann, J.C., Chusid, M.J., Herzig, G.P., and Gallin, J.I.: Functional abnormalities of human neutrophils collected by continuous flow filtration leukopheresis. Blood 46: 901-911, 1975.

8. Gallin, J.I.: The role of chemotaxis in the inflammatory-immune response of the lung. In Kirkpatrick, C.H. and Reynolds, H.Y. (Vol. Eds.): Immunologic and Infectious Reactions in the Lung, Vol. 1, Lung Biology in Health and Disease. Claude Lenfant, Exec. Ed. New York, Marcel Dekker, Inc., 1976, Chap. 8, pp. 161-178.

9. Chusid, M.J., Gallin, J.I., Dale, D.C., Fauci, A.S., and Wolff, S.M.: Defective polymorphonuclear function in a boy with recurrent pyogenic infection. Pediatrics. In press.

I N D E X

LABORATORY OF INFECTIOUS DISEASES

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SUMMARY STATEMENT - Annual Report of the

Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
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The research programs of LID continue to be concerned with the natural history and prevention of the three major uncontrolled acute infectious diseases of man--acute respiratory tract disease, viral hepatitis and acute, nonbacterial gastroenteritis. Broad advances were made in each of these areas during 1975-1976.

Hepatitis

Hepatitis A: A new solid phase radioimmunoassay for the detection of hepatitis A viral antigen and antibody was developed. Using this and other techniques hepatitis A viral antigen was purified from feces of patients acutely ill with type A hepatitis. This antigen was employed in an immune adherence hemagglutination test, modified in this laboratory for use with small quantities of feces-derived hepatitis A antigen, for seroepidemiologic studies of hepatitis A virus infection. In collaborative studies with Bureau of Biologics personnel, San Diego Naval Recruit Center personnel, Dr. James Mosley, Dr. Wolfe Szmuness, Dr. Ian Gust, Dr. Robert McCollum and Dr. Fred Davenport, new knowledge about the ecology of hepatitis A virus was obtained. This included the relationship of age, sex, socioeconomic status, and ethnic and racial origin to the prevalence of hepatitis A virus infection, a preliminary evaluation of the importance of hepatitis A virus in the etiology of "sporadic" (non-epidemic) viral hepatitis in the United States and Australia, laboratory confirmation that hepatitis A virus is important in the etiology of shellfish-associated hepatitis and hepatitis associated with the handling of newly-caught chimpanzees, and the etiologic association of hepatitis A virus with the largest reported epidemic of food-borne hepatitis and two additional smaller outbreaks of viral hepatitis, one occurring in a "commune" and the other in a foundling home. These studies provide the first in-depth analysis of laboratory-confirmed type A hepatitis.

Additional characterization of hepatitis A viral antigen from various sources, including feces, bile and liver, has been carried out. Two populations of hepatitis A virus-like particles were found to exist in most fecal specimens; these had buoyant densities in cesium chloride of, respectively, 1.34 and 1.40. In contrast, hepatitis A antigen derived from liver or bile had a density of 1.34. Anti-serum prepared against the "heavy" and "light" populations of hepatitis A antigen particles from feces confirmed that these particles were indeed antigenically related if not identical. The classification of hepatitis A virus remains in doubt. To resolve this, hepatitis A virus has been purified and is currently being subjected to peptide and nucleic acid analysis. The results of these studies should reveal whether hepatitis A virus is a picornavirus, a parvovirus or a member of a new class of viruses. A detailed study of hepatitis A virus infection in chimpanzees has been carried out. In addition to an analysis of virologic and immunologic indicators of infection, the pathology of the disease as determined by light microscopy and electron microscopy has been analyzed in collaborative studies with Dr.

Hans Popper and Dr. Fenton Schaffner. Hepatitis type A in the chimpanzee could be distinguished from hepatitis type B by light microscopy, the first time such a pathologic distinction has been reported. Attempts are currently in progress to determine if a distinguishing pathologic lesion characterizes human viral hepatitis types A and B. Viral hepatitis types A and B could also be differentiated under code by electron microscopy.

Hepatitis B: Recently an additional hepatitis B antigen, e antigen, and antibody to it were discovered in Sweden. This antigen appears to be virus-specified and has been thought by some to be associated specifically with the Dane particle and long filaments. However, in recent studies with Dr. Gerin, it has been shown unequivocally that e antigen is not on the surface of Dane particles or long filaments and that it is serologically unrelated to hepatitis B surface or core antigens. The main significance of e antigen at present is that its presence in serum appears to be associated with the presence of hepatitis B viral DNA polymerase and a relatively high degree of infectivity. In a recent collaborative study with personnel of the Clinical Center Blood Bank and Dr. Leonard Seeff of the VA, such an association of e antigen with infectivity was confirmed with coded specimens obtained from the recently completed VA hepatitis B hyperimmune globulin (HBIG) study: patients who were inadvertently stuck with a needle contaminated with HB_s Ag positive blood that was also e antigen positive developed type B hepatitis, whereas those stuck with a needle contaminated with HB_s Ag positive but e antigen negative blood did not develop hepatitis. Surprisingly, this association was almost perfect, regardless of whether HBIG or standard immune serum globulin was administered. The findings of this study raise serious questions as to the interpretation of the recently completed HBIG studies and suggest that more extensive serologic evaluation of specimens collected from these studies must be carried out before definitive answers as to the efficacy of hepatitis B immune serum globulin can be obtained.

Recently developed in vitro tests for hepatitis B viral DNA polymerase and hepatitis B core antigen have been applied to a more intensive examination of subpopulations of Dane particles in a collaborative study with Dr. Gerin and Dr. Kaplan. The finding of two populations of Dane particle cores, a "heavy" population (buoyant density = 1.36 in CsCl) and a "light" population (buoyant density = 1.31) has been confirmed by demonstrating two equivalent populations of Dane particles (densities of 1.20 and 1.22) from which the respective heavy and light cores could be extracted. Heavy Dane particles often represent a small proportion of the total Dane particle population. On the basis of these findings and analysis of the clinical characteristics of hepatitis B virus infection (long incubation period, cyclic nature of early antigen development and tendency toward persistence and/or chronicity) we have postulated that populations of hepatitis B viruses may consist of fully infectious virus and defective interfering particles. The presence of defective interfering particles might partially explain the inability to isolate hepatitis B virus in vitro. To explore this further, heavy Dane particles are being carefully purified from the more numerous light particles. When completed, the pools of these particles will be titered for infectivity in chimpanzees and will serve as the inoculum for extensive tissue culture isolation studies.

In collaborative studies with Dr. Gerin, and the Infectious Diseases Branch, approximately 10,000 doses each of monospecific subtype adw and subtype ayw hepatitis B subunit vaccine has been prepared from HB_sAg positive plasma of chronic carriers of the antigen. Immunogenicity tests in guinea pigs have been completed, and both vaccines were found to be highly immunogenic. Safety and efficacy tests of the adw vaccine have begun in chimpanzees and immunogenicity of the vaccine preparation has been confirmed in this species. Tests in chimpanzees of the ayw vaccine will begin shortly. To answer the many questions raised about the safety of such vaccines, extensive tests of humoral and cellular immune response to the vaccine and to subsequent challenge of vaccinated chimpanzees with live virus are being carried out. A micro test for cell-mediated immunity (CMI) to hepatitis B surface antigen is being used to monitor the immune response in vaccinated chimpanzees. Preliminary evidence indicates that chimpanzees respond to the vaccine by the development of both humoral and cellular immunity.

In cooperation with the Infectious Diseases Branch, suitable human populations for initial safety testing of hepatitis B vaccines have been identified, and baseline seroepidemiologic studies are beginning in these groups. It is hoped that within approximately one year the first human trials of these hepatitis B vaccines will begin.

As part of a continuing analysis of our initial hepatitis B vaccine tests in chimpanzees, a previously vaccinated chimpanzee that was found to be refractory to infection with hepatitis B virus when challenged six months after vaccination was rechallenged eighteen months after vaccination: the animal was still fully resistant to infection. This and other vaccinated chimpanzees will be rechallenged thirty months after the initial vaccination, and the results of this experiment will be available before the first human trials of hepatitis B vaccine.

Non-A, Non-B Hepatitis: Diagnosis of non-A, non-B hepatitis is one of exclusion, but the availability of sensitive serologic tests for hepatitis A virus and hepatitis B virus infection has permitted the identification of such non-A, non-B cases. In collaborative studies with Dr. Mosley, Dr. Gust and Clinical Center Blood Bank personnel, non-A, non-B hepatitis was found to account for up to 25% of "sporadic" (non-epidemic) clinical hepatitis seen in Los Angeles and Melbourne, Australia and for up to 90% of clinical and subclinical posttransfusion hepatitis occurring at the Clinical Center Blood Bank. Attempts to identify an antigen analogous to hepatitis A antigen or hepatitis B antigen but specific for non-A, non-B hepatitis have been unsuccessful to date, as have attempts to transmit this agent to chimpanzees and approximately a dozen species of other non-human primates.
(Purcell, Dienstag, McAuliffe, Swiderska, Shimizu)

Viral Gastroenteritis

Recently, important advances have been made in elucidating the etiological agents of acute infectious nonbacterial gastroenteritis, a syndrome which affects a broad segment of the population. It appears that viral gastroenteritis is comprised of at least two entities with distinct epidemiological characteristics. One, which could be designated epidemic

viral gastroenteritis, tends to occur in family or community-wide outbreaks affecting adults, school age children, family contacts and probably young children as well. The illness is usually self-limited and mild, and characteristically lasts 24-48 hours. Previously, we demonstrated by immune electron microscopy (IEM) that a 27 nm particle -- the Norwalk agent -- was associated with an outbreak of this form of gastroenteritis which occurred in Norwalk, Ohio. In addition, particles which resemble the Norwalk agent morphologically have have also been associated by IEM with 2 family outbreaks of gastroenteritis, one in Hawaii and the other in Montgomery County (MC), Md. The Norwalk and Hawaii agents appear to be distinct whereas the Norwalk and MC agents appear to be related.

The other and second form of gastroenteritis, which could be designated sporadic infantile gastroenteritis, has been associated predominantly with a severe form of diarrhea affecting infants and young children, and often requires hospitalization and parenteral fluid therapy. The illness rate among family contacts of infants with this form of diarrhea is low. It is evident now from studies in many parts of the world that a 70 nm human reovirus-like (HRVL) agent, (also designated rotavirus, duovirus, and infantile gastroenteritis virus), is the major etiological agent of sporadic infantile gastroenteritis. Previously, the etiology of the majority of cases of infantile gastroenteritis was unknown. The focus of our gastroenteritis studies during this past year has been on the reovirus-like agents.

Reovirus-like agents - The 5 agents belonging to this group, i.e., the HRVL agent, Nebraska calf diarrhea virus (NCDV), epizootic diarrhea of infant mice (EDIM) virus, simian agent SA-11 and the "O" (Offal) agent, were found to be similar, if not identical, in reciprocal complement-fixation (CF) tests employing hyperimmune animal sera. In addition, in CF tests with paired sera from 35 diarrhea patients who shed the HRVL agent, 74% developed serologic evidence of infection with HRVL antigen, 43% with NCDV, 51% with EDIM virus, 57% with SA-11 and 71% with the "O" agent. Thus, in addition to the NCDV, which we had previously described as a suitable substitute CF antigen for the HRVL agent, the SA-11, "O" and EDIM viruses may also be utilized as substitute antigens for the HRVL agent. However, the "O" agent appears to be the most efficient of the 4 substitute CF antigens and thus should be used preferentially when the HRVL agent is not available. The finding of additional substitute CF antigens for the HRVL agent may have implications in the immunoprophylaxis against human infantile gastroenteritis caused by the HRVL agent. Thus, efforts to find suitable strains of reovirus-like agents in various species should be pursued as it may be possible to immunize against the human disease utilizing an animal reovirus-like strain if one of these strains proves able to infect man without causing illness.

In collaborative studies with Drs. Parrott, Kim and Brandt on the etiology of nonbacterial gastroenteritis among infants and young children admitted to Children's Hospital (D.C.), the HRVL agent was found to be the major etiologic agent of acute diarrheal illness in hospitalized infants and young children. The virus was detected by electron microscopy (EM) in the stools (or rectal swabs) of 42% of 143 patients admitted with acute gastroenteritis between Jan. 1974 and June 1975. Detection of the HRVL agent by electron microscopic examination of stools was extremely efficient. Fifty percent of the patients

studied by both virus detection (EM) and serologic techniques demonstrated evidence of infection with this agent. Infection followed a striking seasonal pattern as the HRVL agent was shed by 59% admitted during the cooler months (Nov.-April), with a peak of 78% in Dec. 1975 and Jan. 1975 combined. In contrast, none of the patients admitted during the warmer months (May-Oct.) shed the agent. Virus-like particles approximately 27 nm in size were observed in stools only infrequently, and their significance is not known at present since they might represent Norwalk-like particles or enteroviruses. None of 275 Eschirichia coli isolates from rectal swabs of 32 study patients with diarrhea were found to produce heat labile enterotoxin by the Y1 mouse adrenal cell assay; 17 (53%) of the 32 patients had evidence of infection with the HRVL agent. Thus, the HRVL agent was the major etiologic agent of diarrheal illness in infants and young children during the cooler months of the year. In serologic studies, it appeared that serum antibody was not the primary determinant of protection against HRVL induced disease.

In an attempt to study the possible mode of transmission of the HRVL agent we studied parental contacts of patients admitted to Children's Hospital with a diarrheal illness. Paired sera from 40 parents of 37 patients infected with the HRVL agent were available for study. Thirteen (32.5%) of the 40 parents developed a serologic response to the HRVL agent and/or the related NCDV. One parent who did not develop a CF antibody response shed the HRVL agent and also developed a serologic response detected by indirect immuno-fluorescence. Thus, infection was detected in 35% of 40 parents of 37 pediatric patients who were admitted to the hospital with HRVL agent disease. Three of the 14 infected parents developed gastrointestinal symptoms at or around the time of their child's illness. The finding of HRVL infection in parents of HRVL infected children suggests a possible mode of transmission of this agent to the very young infant who is often not in contact with other infants and young children.

The response of older individuals to the HRVL agent (HRVLA) was studied further in 10 adult volunteers who were given a particle containing stool filtrate by the oral route. Two of 6 volunteers with a moderately high pre-existing antibody titer for HRVLA and NCDV were infected but did not become ill. Three of the 4 individuals with a low serum antibody titer to HRVLA (CF, IFA) and/or NCDV (neutralization) were infected and 2 of the 3 developed definite illness. In one instance gastroenteritis was observed. It was difficult if not impossible to find adults without pre-existing antibody to the HRVL or related agents. However, the fact that illness could be induced in some adults with little pre-existing antibody is encouraging for the future since the safety and efficacy of vaccine candidate strains can be studied initially in adult volunteers.

The importance of HRVLA in serious infantile diarrhea in other parts of the world was indicated during a study of such disease in rural Bangladesh. In a collaborative study with Dr. R. Ryder of CDC, 48 patients with dehydration secondary to diarrhea who were admitted to a rural Bangladesh hospital were examined for infection with HRVLA or enterotoxigenic Escherichia coli (ETEC). Twelve patients, all less than 2 years of age, had evidence of HRVLA infection; this accounted for 55% of illness in that age group. None of these 22 children had evidence of ETEC infection. Eleven cases of ETEC

diarrhea were detected among the 18 patients who were over 10 years of age. The data suggest that in Bangladesh HRVLA is a common cause of diarrhea in infants and very young children, whereas ETEC is a common cause of diarrhea in older children and adults.

Last year we reported the isolation and 10 successful passages of a strain ("Fh") of HRVLA in human embryonic kidney (HEK) cell culture. We have now carried this virus strain for a total of 16 serial passages. In higher passages small numbers of virus-like particles were visualized by electron microscopy of negatively stained preparations. Of interest, these particles were incomplete or subviral, lacking an outer capsid. At the 13th passage level ultrathin sections of infected cells were examined by electron microscopy, and reovirus-like particles were seen in vesicles bounded by rough endoplasmic reticulum. When high passage level cultures were examined by indirect immunofluorescence small numbers (usually less than 1%) of cells were observed to contain viral antigens. To establish the specificity of these antigens tests were performed with acute and convalescent sera from 2 patients who had gastroenteritis associated with HRVLA. Both patients developed antibody to the antigens in cell culture. Also hyperimmune guinea pig serum prepared against concentrated and purified HRVLA produced fluorescent staining while the pre-immunization serum from the same animal did not. These experiments indicate that cultivation of HRVLA was achieved in human embryonic kidney cell cultures. However, since other viruses which are currently indistinguishable serologically from the human virus were also under study, the possibility of a laboratory contaminant must be considered. However, 3 of the related viruses (the NCDV, the SA-11 and the "O" agent) are adapted to cell culture and produce cytopathic effects in either bovine embryonic kidney or African green monkey kidney culture whereas the strain of virus isolated in HEK cells does not.

In continuing studies of HRVLA in newborn rhesus monkeys the agent was passaged successfully 5 times in series but illness occurred only during the first passage. The absence of diarrhea during the second and succeeding passages suggests the possibility of rapid attenuation in a nonhuman primate. Since one of our goals is the development of an attenuated vaccine strain these observations may be of some significance. Efforts to induce disease in rhesus monkeys after weaning met with failure although infection did occur.

Gnotobiotic newborn calves administered the HRVLA did not develop diarrhea, but were infected as indicated by an immunologic response and shedding of virus in stools. During the second passage in calves, illness developed. Virus was serially passaged 3 additional times in newborn calves and at each passage level diarrhea was observed. Five gnotobiotic calves inoculated at 3-5 days of age, did not develop diarrheal illness indicating a rather strict age-resistance effect. Intestinal lesions induced by third and fourth calf passage HRVLA were studied in gnotobiotic calves using light and immunofluorescence microscopy, and scanning and transmission electron microscopy. The sequence of events in the small intestine was: (1) infection of the absorptive villous epithelial cells; (2) replacement of the tall columnar villous epithelial cells with cuboidal and squamous cells, shortening of the villi, enlargement of reticular cells and lymphocytic infiltrations into the villous lamina propria; and (3) repair.

Further studies were undertaken in gnotobiotic newborn piglets which were demonstrated last year to be susceptible to HRVLA with the development of a generally self-limited diarrheal illness. Thus far we have not been able to induce diarrheal illness in older gnotobiotic piglets. None of 7 animals challenged at 3 to 5 weeks of age with virulent HRVLA developed diarrhea, although 4 were infected.

The availability of a large quantity of calf stool containing the human reovirus-like (HRVL) agent provided an opportunity to perform biophysical and biochemical studies with this agent and made possible a comparison with related reovirus-like agents. RNA was extracted from purified virus by treatment with phenol-chloroform (1:1) and then precipitated in ethanol. By polyacrylamide gel electrophoresis of RNA it was possible to distinguish between the human and calf reovirus-like agents associated with diarrhea. These 2 viruses differed in as many as 5 of their RNA genome segments on the basis of electrophoretic mobility on 9% gels. In addition, comparison of the RNA segments of two other related viruses (the "O" agent and SA-11 virus) indicated that these viruses differed from the human and calf viruses as well as from each other. Thus, for the first time it was possible to distinguish the members of the reovirus-like agent group by a laboratory technique. This method will be applied to the identification of the virus which has been propagated in human cell culture and which we believe to be HRVLA. Each of the 4 viruses of the group appeared to possess 11 double stranded RNA segments in contrast to the reoviruses which have 10 RNA pieces. Based on contour length measurement, the RNA segments of the human and calf viruses were divided into 4 size classes in contrast to the 3 size classes seen with the true reoviruses. (Kapikian, Wyatt, VanKirk, Kalica, Chanock)

Influenza virus

Efforts have continued to develop and define temperature sensitive (ts) genetic lesions which effect attenuation of any influenza A virus into which they are transferred. This approach is based upon our experience during the past few years which indicates that ts mutation produces a restriction of viral replication which is temperature dependent. Thus, ts mutants are more restricted in growth in the lungs of hamsters, where the temperature is 37°C, than in the nasal turbinates, where the temperature is 32°C. In this manner replication is restricted in the lower respiratory tract which is the major site of pathology, whereas virus can grow to moderate titer in the cooler upper air passages and thereby stimulate local and systemic immunologic defense mechanisms.

Specification of attenuation by defined genetic lesions which can be assayed for in the laboratory offers an advantage to the use of ts mutants for vaccination. Thus, ts mutants used as vaccine strains can be assessed for genotype and genetic stability during vaccine development, vaccine production and later during infection of man. In this manner the genetic basis for

attenuation can be monitored directly during all phases of vaccine development, manufacture and usage.

The 2 ts-1[E] lesions (complementation groups 1 and 2) are of particular interest since they have been transferred into 3 succeeding antigenic variants of the H3N2 subtype and such ts recombinants have been evaluated extensively in animals and in man. Previously, ts-1[E] recombinants bearing the H3N2 surface antigens of the Hong Kong/1968 and Udorn/1972 (identical to London/1972) variants were evaluated in adults who lacked serum antibody for the H3 hemagglutinin antigen but possessed serum antibody for the N2 neuraminidase antigen. In these circumstances the ts-1[E] recombinants were satisfactorily attenuated, antigenic, stable genetically, non-communicable and highly protective. During the past year a more contemporary ts-1[E] recombinant bearing the surface H3N2 antigens of the Georgia/1974 virus (equivalent to Port Chalmers/1974) was evaluated in adult volunteers who lacked serum antibody for H3 but possessed N2 immunity. This recombinant had a 38°C shutoff temperature for plaque formation in tissue culture and was moderately restricted in its replication in the lower respiratory tract of the hamster. In these properties it was identical to the 2 preceeding ts-1[E] recombinants evaluated in man. Thirty-two volunteers were given $10^{6.5}$ $TCID_{50}$ of the Georgia ts-1[E] recombinant and 94% became infected. The virus was suitably attenuated and stimulated a moderately high level of serum and local respiratory tract antibodies. Furthermore, each of the isolates recovered from the 23 adults who shed virus retained the ts phenotype, indicating that the recombinant was stable genetically in individuals with some neuraminidase immunity. Thus, the most recent ts-1[E] recombinant appeared to be identical to the 2 preceeding ts-1[E] recombinants. This observation provides support for the view that any virus within the H3N2 subtype that possesses the 2 ts-1[E] lesions and a 38°C shutoff temperature for plaque formation will be suitably attenuated and antigenic for adults who possess some neuraminidase immunity.

Although the ts-1[E] lesions render recombinant viruses satisfactorily attenuated for individuals with some neuraminidase immunity, these lesions may not be suitable for recombinants which are to be given to persons who lack immunity to both surface antigens of the virus, i.e., doubly seronegative individuals. Previously 2 ts-1[E] recombinants were shown to induce a transient febrile response in doubly seronegative young children and for the first time evidence of genetic instability (reversion to the ts^+ or wild type phenotype) was observed. These observations suggested that 2 types of ts lesions may be required for production of ts recombinants to be used for vaccination. The ts-1[E] lesions could be recommended for production of ts recombinants to be used in older children and adults during interpandemic intervals when such individuals possessed some neuraminidase immunity. For immunization of doubly seronegative persons, i.e., young children at all times and all members of the population at the time of emergence of a new pandemic subtype such as HSw1 N1 or H4N3, a set of ts lesions which are more restrictive than ts-1[E] would be needed.

During the past year several ts lesions which might meet this need were characterized. The influenza A/Hong Kong/1968 ts-1[A] recombinant was shown previously to be more restricted than the Hong Kong/1968 ts-1[E] in hamsters and in man. The two ts lesions of this recombinant, located in complementation

groups 1 and 5, were transferred to the Udorn/1972 virus in order to evaluate their restrictive effect in another recombinant. This recombination effort yielded 2 classes of Udorn recombinants--one class possessed both group 1 and group 5 lesions while the other class possessed only the group 1 lesion. In mice, the single recombinants, typified by clone 189, were as restricted in growth and stable genetically as the double lesion recombinants, respresented by clone 46. Both clone 189 and clone 46, however, were more restricted and more stable in mice than ts-1[E]. Thus, it appeared that the group 1 lesion of ts-1[A] was the major determinant of its level of defectiveness.

The group 1 lesion of ts-1[A], as segregated into clone 189, was combined with the group 5 ts lesion of the 315 ts mutant of Hong Kong virus by mating clone 189 and ts-315 and selecting for a double recombinant possessing both ts lesions. This double lesion recombinant was produced because the 315 group 5 lesion had been shown previously to be quite restrictive when acting by itself. Thus, the new double recombinant, designated 1A2, contained 2 ts lesions, each of which was quite restrictive in its effect on replication in the lungs. Tests in animals confirmed that the 1A2 double recombinant was more restricted in growth in the lungs than was ts-1[E].

Three donors of ts lesions were used to produce ts recombinants of the influenza A/Victoria/1975 virus: a) ts-1[E] (group 1 and group 2 lesions), b) clone 189 (group 1 lesion) and 3) 1A2 (group 1 and group 5 lesions). The ts-1[E] recombinant of A/Victoria/1975 has been completely characterized and a suspension of virus to be evaluated in volunteers is currently undergoing extensive safety tests. The other 2 Victoria recombinants have been prepared and will follow a similar progression shortly. Efforts to produce ts recombinants of influenza A/New Jersey/1976 "swine flu" virus were begun after the virus emerged as a potential pandemic threat. ts-1[E] recombinants have been produced and 189 and 1A2 recombinants will be produced in the near future. During the next year the 3 classes of ts Victoria recombinants will be evaluated in volunteers and hopefully in the field. The swine influenza recombinants will be evaluated in volunteers under strict conditions of isolation. Studies with the swine ts recombinants in open populations will not begin until the virus has established an early epidemic pattern.

Sugiura, Kilbourne, Ueda, Tobita, Krug and their collaborators have developed and characterized a series of 5 FU ts mutants of the WSN strain of influenza A virus. These mutants have been divided into 7 complementation groups by genetic analysis and the biochemical defect of 6 of the groups has been established. We sought to determine the biochemical defect in members of each of our 7 complementation groups by carrying out a genetic comparison between the NIH Hong Kong and WSN sets of 5 FU ts mutants. On the basis of this comparison we established that HK 1 = WSN 1, which has a defect in C RNA; HK 6 = WSN 2, which has a defect in V RNA; and HK 5 = WSN 3, C RNA defect. It is of interest that the 2 ts lesions (group 1 and group 5) which are most promising for use in vaccines both affect the same early function, namely transcription of viral RNA into complementary RNA. Three of the WSN groups i.e., 4 (neuraminidase defect), 6 (hemagglutinin defect) and 7 (defect unknown) complement all 7 Hong Kong groups. Likewise 4 of the Hong Kong groups (2,3,4,7) complement all 7 WSN groups. WSN group 5 complements with Hong Kong groups 1, 3, and 5, but does not appear to complement with the

other 4 groups at a high level. If a correspondence can be demonstrated between the WSN group 5 and one of the Hong Kong groups then the total number of complementation groups accounted for by the 2 sets of mutants would be 10, a number which approximates the number of unique pieces of viral RNA demonstrated by polyacrylamide gel electrophoresis. The Hong Kong and WSN groups with unassigned function may correspond to genetic material coding for membrane protein, nucleocapsid or non-structural protein assuming that none of these proteins also has a role in V RNA or C RNA synthesis. The absence of Hong Kong mutants with ts lesions in the genes controlling the hemagglutinin and neuraminidase glycoproteins had been suggested by our previous findings using a marker rescue technique.

Comparison of our ts mutants and the cold adapted (CA) viruses and their cold recombinants (CR) developed by Maassab, Kendal and Davenport has continued. Six independently derived CA viruses and 4 sets of cold recombinants derived from one CA parent were studied for ts lesions. Four of the CA viruses were ts as were 3 sets of recombinants. Each ts CA virus possessed the group 1 lesion and 3 of the CA viruses had additional lesions. Ten recombinants obtained from one cross between a CA parent (A/AA/60) and Scotland/74 were each ts and each recombinant contained only the group 1 ts lesion.

The genetic findings suggest that the cold adaptation property might be linked in some manner to the group 1 lesion. In order to ascertain whether this was so we studied the ts and cold adaptation properties of 2 sets of viruses: (i) revertant viruses with the ts^+ phenotype isolated from hamsters and (ii) recombinant viruses prepared at 34°C using the wild type influenza A 1943 (HON1) Marton virus as one parent and the CR12 virus (with group 1 ts lesion) as the other. Among these virus populations we identified viruses with various combinations of the 2 properties: ts^+ recombinants which were cold adapted and some which were not; revertant ts^+ viruses which were no longer cold adapted and some which retained the cold adaptation property. Thus the ts and cold adaptation mutations may not be the same, but this issue requires further study. It is clear, however, that selection for CA mutants often co-selects for ts mutations especially in complementation group 1.

CA recombinants were evaluated in hamsters for growth pattern in the respiratory tract and for genetic stability. The findings were in accord with previous results in that the restriction of growth in the hamster lung correlated with the shutoff temperature, i.e., the lower the shutoff temperature—the greater the restriction. Cold adapted viruses were not inherently more stable genetically in vivo than ts recombinants with a similar level of temperature sensitivity. The cold recombinants CR 12 and CR 18-6, with a shutoff temperature of 38°C, were more stable in vivo than the Hong Kong/68-ts-1[E] virus which also shutoff at 38°C. However, the Udorn/72-ts-189 recombinant was more stable (i.e., revertants were not detected) than the CR18-7 although both recombinants had a 37°C shutoff temperature for plaque formation. (Murphy, Spring, Shimizu, Chanock)

Respiratory Syncytial Virus

Efforts in the RSV program this year were directed toward the following goals: (1) a more precise definition of the genetic makeup of the RS virus

genome through grouping of ts viruses into appropriate complementation groups; (2) further study of the genetic stability of the ts mutants in an effort to define the conditions under which a virus is most genetically unstable and thereby develop new criteria for the evaluation of ts mutants as potential candidates for use in immunoprophylaxis of RS virus disease in infants; (3) further examination of the phenotypic expression of the ts viruses; (4) evaluation of the growth and pathogenesis of RS virus in animal models.

Previously ts mutants were grouped into 3 complementation groups based on their ability to complement one another at the restrictive temperature of 38° or 39°C. Group A consists of ts-1, ts-3, ts-4, ts-5, and ts-6. Groups B and C consist of single representatives, ts-2 and ts-7 respectively. In addition ts-2 is a plaque morphology mutant, since it forms atypical plaques which lack syncytia. Based on the probable molecular weight of the RS virus genome, the existence of at least 6-7 complementation groups is likely. We therefore attempted to group other existing ts mutants into complementation groups. Three new ts mutants produced at Flow Labs in earlier years, ts-8, ts-9, and ts-10, were tested for complementation. Ts-9, ts-10, and ts-11 were clearly group A mutants since they did not complement with ts-1 but did so with ts-2 and ts-7. In addition, the ts-1 NTG 1 and ts-1 NTG 16, produced by subjecting ts-1 to an additional cycle of mutagenesis with nitrosoguanidine (NTG) were studied for the nature of the additional ts lesions induced by NTG. The ts-1 NTG 1 and ts-1 NTG 16 retain their ability to complement with group B and group C mutants but do not complement with group A mutants. This indicates that the additional ts lesion or lesions are not in the region of group B or group C genes.

Methods for increasing the emergence of ts^+ revertants were developed so that they could be applied to the evaluation of candidate vaccine strains. To date the ts-1 NTG 1 appears to be the most defective and most stable of the RS mutants and this strain will be used to prepare a suspension of virus which will be evaluated in adults and later in children.

The possibility that a live attenuated RSV vaccine might induce a state of altered reactivity to RSV infection, similar to that observed previously with inactivated RSV vaccine, has been raised on a number of occasions. During the past year the response of RSV vaccinees to RSV infection was analyzed in an attempt to answer this question. This study was performed in collaboration with Drs. Parrott and Kim of Children's Hospital of D.C. A total of 13 initially serum antibody negative infants received either the live 26°C adapted RSV vaccine (3 infants) or the live ts-1 mutant (10 infants). Following vaccination 11 of the 13 vaccinees underwent natural infection with RSV on one or more occasions during subsequent annual RSV epidemics. Twelve of the infections were inapparent and the one child who became ill during RSV infection had only an upper respiratory illness with a maximum fever of 100°. Thus there was no evidence that the 2 live attenuated RSV vaccines induced a state of altered reactivity to infection. (Richardson, Belshe, Chanock)

Streptococcus pneumoniae

Although parenterally administered capsular polysaccharide vaccine is highly effective in preventing pneumococcal pneumonia in adults, it may be less effective in preventing otitis media in infants. Thus, there is a need to explore alternate approaches to prevention of this type of ear disease. We have selected ts mutants of S. pneumoniae which grow vigorously at 32°C, but which do not replicate efficiently at 38°C. Mutants of this type should replicate in the nasopharynx and stimulate local antibody, but should not produce disease in the middle ear or lower respiratory tract because of their failure to grow to high titer at the restrictive temperature of these areas. Ts mutants of type 1 S. pneumoniae were selected following exposure of virulent (ts^+) organisms to nitrosoguanidine. Each mutant resembled the ts^+ parent in bacteriologic and serologic properties, but was restricted in capacity to form colonies at 38°C. When inoculated intraperi-toneally (IP) into mice, 11 of 13 mutants were attenuated and induced homologous resistance. Each of 3 mutants studied was also attenuated and immunogenic following administration IP to hamsters. Attenuation in hamsters appears to be related to decreased growth and survival of ts organisms at body temperature. The 3 mutants studied were completely attenuated for hamsters when administered intranasally and 2 of the mutants induced resistance to subsequent challenge with ts^+ organisms by the same route. (Helms, Grizzard)

Mycoplasma pneumoniae

Employing a constant dose of organisms the influence of particle size and route of administration of M. pneumoniae on the course of experimental infection was studied in hamsters. Large particle aerosols (10 um) were deposited primarily in the upper respiratory tract (URT), tended to remain in this area, and did not induce significant pathology in the lungs. In contrast, small particle aerosols (2.4 um) were distributed throughout the respiratory tract and were associated with the development of pulmonary lesions. The small particle aerosols were more efficient in initiating infection. Both types of aerosol induced significant resistance to subsequent intranasal challenge with virulent M. pneumoniae, but the small particle aerosol was more effective in this regard.

Hamsters could be infected by oral administration of virulent M. pneumoniae or one of its ts mutants (ts 640). Hamsters inoculated by this route underwent a silent infection limited primarily to the URT and were significantly resistant to subsequent intranasal challenge with virulent M. pneumoniae.

In an effort to establish an experimental disease with a closer resemblance to human disease 3 rhesus monkeys were inoculated via the URT with 10^7 CFU. Each animal underwent an asymptomatic infection with a serological response and shedding of the organism. Inoculation of 10^7 CFU in the URT and LRT of 3 squirrel monkeys also resulted in a silent infection. Subsequent challenge of these monkeys was followed by a shorter, more restricted infection without accompanying disease. There was no evidence of transmission of the organism to broth-inoculated cagemate controls.

In initial studies in volunteers, the ts H43 mutant appeared to be satisfactorily attenuated yet antigenic. During the past year more extensive experience

with the mutant in 75 volunteers indicated that it retained a low level of residual virulence hen administered in a dose of 10^6 to 10^8 CFU by coarse aerosol intranasally. At this dose level almost all seronegative volunteers (serum MI antibody titer $\leq$ 1:2) became infected. Several volunteers developed a mild afebrile bronchitis. Two modifications in mode of administration were evaluated in an attempt to eliminate this residual virulence. First, a smaller dose of organisms, 10^4 - $10^{4.9}$ CFU, was given, but this was not sufficient to infect a majority of volunteers. Second, the ts H43 mutant was administered by the oral route in a dose of 10^6 CFU, but only one of 7 volunteers became infected.

The ts H28 mutant was also studied in volunteers. This mutant is more temperature sensitive and defective than ts H43. Consonant with these in vitro properties the ts H28 mutant was found to have low infectivity for volunteers. Only 8% of 23 individuals were infected when given 10^6 CFU intranasally. (Grizzard, Helms, Chanock)

Mycoplasmas

Studies on the suckling mouse cataract agent (SMCA) have been extended in collaboration with Dr. Fred Clark (Wistar Inst.) and Dr. Robert Whitcomb (USDA). SMCA, and a second tick-derived agent (GT-48) were grown in the developing chick embryo and both allantoic and amniotic fluids contained numerous motile helical forms typical of spiroplasmas (wall-free prokaryotes previously found only in plant diseases or insect vectors). This finding offered the first important evidence that spiroplasmas were not confined to plants or insects and that they could be pathogenic for vertebrates. The occurrence of a new class of microbial agent not capable of being cultured on conventional laboratory media, nor detected by the usual serological tests with other microbial forms, suggested a search for these agents in human diseases of unknown etiology. SMCA or GT-48 isolates were successfully cultivated on 2 of 20 different media formulations prepared from conventional mycoplasma broth supplemented with various tissue culture media components. Growth did not occur in conventional mycoplasma broth alone, nor in other media used for spiroplasmas. Titers of 10^8 to 10^9 color-changing units/ml were obtained after several passages in artificial media. Virulence tests in the chick embryo with cultured SMCA or GT-48 strains revealed egg LD_{50} titers of greater than $10^{9.0}$/0.5 ml culture medium. Cultured SMCA induced central nervous sytem disease and death in 50% of suckling rats inoculated intracerebrally. All survivors have exhibited bilateral cataracts. Consistent recovery of SMCA spiroplasmas was accomplished in artificial media from rats challenged with cultured SMCA, thus documenting Koch's postulates on the etiological relationship between the occurrence of cataracts and CNS symptoms to the cultured agent.

A complement-fixation test, using cultured SMCA antigen, has been employed in sero-epidemiological studies on selected normal human sera and paired sera of children and young adults exhibiting a variety of CNS diseases of unspecified etiology. Approximately 50% of normal human sera have shown low levels (1:4 to 1:8) of antibody to SMCA. Paired sera from 10 of 47 children with CNS disease (mostly aseptic meningitis) revealed 4-fold or greater increases in SMCA antibody. CF tests on a group of sera from children with confirmed Reye's syndrome did not point to any consistent association

between antibody levels to SMCA and occurrence of disease. Additional CF tests are planned with other human sera and with techniques to increase the specificity and sensitivity of the serological test system.

Preliminary studies on primate pathogenicity of cultured SMCA have been performed in cynomolgus monkeys. Early results indicate some CNS disease resulting from a variety of challenge routes and always associated with a serological response to SMCA. The occurrence of fairly high (1:128) pre-existing SMCA antibody levels in normal cynomolgus monkeys (although not apparent in normal rhesus monkeys) suggests the need for further study of the origin of this antibody and its role in the observed challenge responses. (Tully)

Honors and Awards

Robert M. Chanock

Advisor, Scientific Group on Virus Diseases, WHO, Geneva, Sept., 1975
Co-chairman, Symposium on Viruses of the Respiratory Tract, International Congress of Virology, Madrid, Spain, September, 1975
Chairman, Session IV at Symposium on Recent Advances in Bacterial and Viral Gastroenteritis, Medical College of Virginia, Richmond, Virginia, February, 1976
Recipient, Modern Medicine's Distinguished Achievement Award, 1976
Elected Chairman, Infectious Disease and Immunology Section, National Academy of Sciences, 1976
Invited speaker, Boston Infectious Disease Dinner Group (Harvard, Tufts, Boston University), February, 1976
Invited speaker, Seminar, University of Colorado, Genetic Manipulation of Influenza Virus for Purpose of Immunization, April, 1976
Member, Virus Cancer Program Scientific Review Committee B, National Cancer Institute
Member, Ad Hoc Study Group R & D Command, U.S. Army Department of Defense, Walter Reed Army Institute of Research

Albert Z. Kapikian

Invited to present seminar on hepatitis and gastorenteritis at Georgetown School of Medicine, Department of Microbiology, May 31, 1975.
Invited participant to Airlie Conference on Crohn's Disease: National Cooperative Crohn's Disease Study Data and Future Research Directions, Airlie, Virginia, June 29 - July 1, 1975.
Invited Speaker at Official Opening Ceremony of the World Health Organization Collaborating Centre for Collection and Evaluation of Data on Comparative Virology Oct. 15-17, 1975 in Munich, West Germany. Presented paper on Oct. 16, 1975 on "Reovirus-like Agents" as the Discussant for Reovirus.
Invited speaker at the Third International Congress for Virology, Madrid, Spain Sept. 10-17, 1975. Presented paper on Sept. 10, 1975 "Studies of the Human Reovirus-like Agent Associated with Infantile Gastroenteritis" at Workshop on Clinical Virology.
Invited speaker at The Ciba Foundation Symposium on Diarrhea in Childhood, Oct. 20-23, 1975; in London, England. Presented paper on Oct. 23, 1975 "Further Studies of the Reovirus-like Agent in Infantile Gastroenteritis".
Invited speaker at D. C. Children's Hospital, Washington, D. C. Infectious Disease Rounds on Oct. 31, 1975. Presentation on Reovirus-like Agents of Infantile Gastroenteritis.
Invited speaker at Pediatric Grand Rounds at Georgetown University Hospital, Jan. 16, 1976. Presentation on Viral Gastroenteritis.
Invited to be Chairman of Session "Specific Viral Agents of Gastroenteritis" on Feb. 6, 1976 at Symposium on "Recent Advances in Bacterial and Viral Gastroenteritis" at Medical College of Virginia, Richmond, Va. Feb. 5-6, 1976.

Invited speaker at Infectious Disease Lecture Series in 11E Solarium of Clinical Center, NIH, March 31, 1976.
Invited speaker at seminar on "Intestinal Pathogens & Mechanisms of Defense" on May 3, 1976 at Annual Meeting of American Society for Microbiology, Atlantic City. Title of presentation listed in program "Reo-like Viral Agents".

Robert H. Purcell

Co-chairman, Workshop on Viral Hepatitis, International Congress of Virology, Madrid, Spain, September, 1975.
Invited speaker, 3rd International Congress of Virology, Madrid, Spain, September 10-17, 1975.
McLaughlin Visiting Professor at the University of Texas Medical Branch, August, 1975.
Member, Ad Hoc Study Group $ & D Command, U.S. Army Department of Defense, November, 1975.
Invited speaker, Symposium on Viral Hepatitis, Society of Medical Consultants to the Armed Forces, December, 1975.
Invited Speaker, Postgraduate continuation course on "Gastroenterology for Clinicians-1976", Phoenix, Arizona, March 18-20, 1976.
Invited participant, panel discussion on "Viral Hepatitis Update" at the 57th Annual Meeting of the American College of Physicians, Philadelphia, Pa., April 5-8, 1976.
Invited speaker of the pediatric group at the 1976 Summit meeting of the Oklahoma State Medical Association, May, 1976.

Joseph G. Tully

Invited speaker, American Soc. for Microbiology seminar on Spiroplasmas, May 6, 1975, New York, N.Y.
Invited speaker, Bureau of Biologics, Division of Bacterial Products, seminar, October 16, 1975.
Invited speaker, Worshop on Mycoplasmas and Cell Cultures, Lake Placid, N.Y. Dec. 8-10, 1975.
Invited speaker, American Soc. for Microbiology seminar on Acholeplasmas and Evolution, Atlantic City, N.J., May 6, 1976.
Invited participant, Symposium on Non-gonococcal Urethritis and Related Oculo-genital Infections, Lake Placid, N.Y., April 28-30, 1976.
Chairman & Member of Board, FAO/WHO Program on Comparative Mycoplasmology.
Chairman, Subcommittee on Taxonomy of the Mycoplasmas, American Soc. for Microbiology.
Member, International Subcommittee on the Taxonomy of Mycoplasmatales.
Member, International Civil Service Committee, American Academy of Microbiology.
Member, Organizing Committee, International Organization of Mycoplasmologists.

Brian R. Murphy

Invited participant, Working Group on Pandemic Influenza, Rougemont, Switzerland, January, 1976.

Richard G. Wyatt

Invited speaker at the Third International Congress for Virology, Madrid, Spain (Sept. 10-17, 1975). Presented paper on Sept. 10, 1975, entitled "27 nm Parvovirus-like Particles Associated with Norwalk Outbreak of Acute Epidemic Nonbacterial Gastroenteritis: Recognition of Similar Particles from Two Other Outbreaks".

Invited speaker at 56th Annual Meeting of the Conference of Research Workers in Animal Disease, Chicago, Ill., Dec. 1-2, 1975. Presented paper on "Human Reovirus-like Agent - Induced Diarrhea in Gnotobiotic Pigs".

Invited speaker at Symposium on Recent Advances in Bacterial and Viral Gastroenteritis, Richmond, Va., Feb. 5-6, 1976. Presented paper entitled "Studies with Reovirus-like Agent Associated with Infantile Gastroenteritis".

Anthony R. Kalica

Invited speaker at Fifteenth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C. Sept. 24-26, 1976. Presented paper on Sept. 25, 1976 entitled "Epidemic Gastroenteritis" at Symposium on Viruses and Diarrhea.

Dale VanKirk

Invited speaker at Fifteenth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C. Sept. 24-26, 1976. Presented paper on Sept. 25, 1976 entitled "Reovirus-like Agent Associated with Infantile Gastroenteritis" at Symposium on Viruses and Diarrhea.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00021-07 LID

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TITLE OF PROJECT (80 characters or less)

The Etiology of Acute Infectious Nonbacterial Gastroenteritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Anthony R. Kalica	NIAID/LID	Microbiologist
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	Dr. Richard G. Wyatt	NIAID/LID	Medical Officer

Other investigators:

Dr. Robert M. Chanock	NIAID/LID	Chief, LID, NIAID
Mr. W. Lee Cline	NIAID/LID	Bio. Lab. Tech

COOPERATING UNITS (if any) LCI, NIAID; Children's Hospital, Wash., D.C.; NINDS, Infectious Diseases Branch; Meloy Laboratories; Dept. Veterinary Sciences, University of Nebraska; Dept. of Medicine, Johns Hopkins University Sch. of Med., Balt., Md.; CDC, Atlanta; LBV, NIAID; LSD, NIAID; MAN Laboratory

LAB/BRANCH Laboratory of Infectious Diseases

SECTION Epidemiology Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
122/12	36/12	86/12

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives - (1) To search for viruses which play an etiological role in the syndrome of acute infectious non-bacterial gastroenteritis of infants, children, and adults; (2) to cultivate (in vitro) the viral agents of acute infectious nonbacterial gastroenteritis; (3) to study the biophysical, immunological and epidemiological characteristics of such agents; (4) to reproduce the syndrome experimentally for the purpose of a) studying the pathophysiological and immunological responses of the host, and b) assaying the infectivity of such viral agents such as reovirus-like agents and the Norwalk and Norwalk-like agents; (5) to develop effective immunoprophylaxis for the viruses of acute, infectious, nonbacterial gastroenteritis.

Major Findings

Background: Recently, important advances have been made in elucidating the etiological agents of acute infectious nonbacterial gastroenteritis, a syndrome which affects a broad segment of the population. It appears that viral gastroenteritis is comprised of at least two entities with distinct epidemiological characteristics. One, which could be designated epidemic viral gastroenteritis, tends to occur in family or community-wide outbreaks affecting adults, school age children, family contacts and probably young children as well. The illness is usually self-limited and mild, and characteristically lasts 24-48 hours. In 1972, we demonstrated by immune electron microscopy (IEM) that a 27 nm particle -- the Norwalk agent -- was associated with an outbreak of this form of gastroenteritis which occurred in Norwalk, Ohio. In addition, particles which resemble the Norwalk agent morphologically have also been associated by IEM with 2 family outbreaks of gastroenteritis, one in Hawaii and the other in Montgomery County (MC), Md. The Norwalk and Hawaii agents appear to be distinct, whereas the Norwalk and MC agents appear to be related.

The other and second form of gastroenteritis which could be designated sporadic infantile gastroenteritis has been associated predominantly with a severe form of diarrhea affecting infants and young children, and often requires hospitalization and parenteral fluid therapy. The illness rate among family contacts of infants with this form of diarrhea is low. It is evident now from studies in many parts of the world that a 70 nm human reovirus-like (HRVL) agent, (also designated rotavirus, duovirus, and infantile gastroenteritis virus), is a major etiological agent of sporadic infantile gastroenteritis. Before the discovery of this agent by Australian investigators in 1973, the etiology of the majority of cases of infantile gastroenteritis was unknown.

The focus of our gastroenteritis studies during this past year has been on the reovirus-like agents. Efforts to detect 27 nm particles in clinical specimens as well as to develop a serological assay (immune adherence) for the Norwalk agent have been made. However, the reovirus-like agents have occupied most of the research time of this section. These studies are described below.

A. Antigenic Relationships Among 5 Reovirus-like Agents by Complement Fixation and Development of New Substitute CF Antigens for the Human Reovirus-like (HRVL) Agent: The HRVL agent, Nebraska calf diarrhea virus (NCDV), epizootic diarrhea of infant mice (EDIM) virus, simian agent (SA)-11 and the "O" (Offal) agent were found to be similar, if not identical, in reciprocal CF tests employing hyperimmune animal sera. In addition, in CF tests with paired sera from 35 diarrhea patients who shed the HRVL agent, 74% developed serologic evidence of infection with HRVL antigen, 43% with NCDV, 51% with EDIM virus, 57% with SA-11 and 71% with the "O" agent. Thus, in addition to the NCDV, which we had previously described as a suitable substitute CF antigen for the HRVL agent, the SA-11, "O" and EDIM viruses may also be utilized as substitute antigens for the HRVL agent. However, the "O" agent appears to be the most efficient of the 4 substitute CF antigens and thus should be used preferentially when the HRVL agent is not available. The

greatest efficiency for detecting infection with the HRVL agent resulted when sera were tested with both the HRVL and "O" agents as 31 (89%) of the patients developed serologic evidence of infection with one or both antigens. The finding of additional substitute CF antigens for the HRVL agent may have implications in the immunoprophylaxis of human infantile gastroenteritis caused by the HRVL agent. Thus, efforts to find suitable strains of reovirus-like agents in various species should be pursued as it may be possible to immunize against the human disease utilizing an animal reovirus-like strain if one of these strains proves able to infect man without causing illness. Of course, attempts to find naturally-occurring attenuated strains of the human agent or to develop such attenuated strains should be pursued as well as attempts to develop inactivated vaccines once the HRVL agent is grown efficiently in cell culture.

B. Studies on the Etiology of Nonbacterial Gastroenteritis Among Infants and Young Children Admitted to Children's Hospital (D.C.): The HRVL agent was found to be the major etiologic agent of acute diarrheal illness in hospitalized infants and young children. The virus was detected by electron microscopy (EM) in the stools (or rectal swabs) of 42% of 143 patients admitted with acute gastroenteritis between Jan. 1974 and June 1975. Fifty percent of the patients studied by both virus detection (EM) and serologic techniques demonstrated evidence of infection with this agent. Detection of the HRVL agent by electron microscopic examination of stools was extremely efficient. Infection followed a striking seasonal pattern as the HRVL agent was shed by 59% of the patients admitted during the cooler months (Nov.-April) with a peak of 78% in Dec. 1975 and Jan. 1975 combined. In contrast, none of the patients admitted during the warmer months (May-Oct.) shed the agent. Virus-like particles approximately 27 nm in size were observed in stools only infrequently, and their significance is not known at present since they might represent Norwalk-like particles or enteroviruses. None of 275 Escherichia coli isolates from rectal swabs of 32 study patients with diarrhea was found to produce heat labile enterotoxin by the Y1 mouse adrenal cell assay; 17 (53%) of the 32 patients had evidence of infection with the HRVL agent. Thus, the HRVL agent was the major etiologic agent of diarrheal illness in infants and young children during the cooler months of the year. In serologic studies, it appeared that serum antibody was not the primary determinant of protection against HRVL induced disease.

C. Studies of Parents of Pediatric Patients with HRVL Infection: In an attempt to study the possible mode of transmission of the HRVL agent we studied parent contacts of patients admitted to Children's Hospital with a diarrheal illness. Paired sera from 40 parents of 37 patients infected with the HRVL agent were available for study. Two of these patients had not been included in the study group previously since stools had not been obtained from them. A parent's initial blood specimen was obtained at or within a few days of admission of his or her child followed by a second blood specimen about 1 month later. Thirteen (32.5%) of the 40 parents developed a 4 fold increase in serum CF antibody to the HRVL agent and/or NCDV. One parent who did not develop a CF response shed the HRVL agent and also developed a response by indirect immunofluorescence. Thus, 14 (35%) of 40 parents of 37

patients infected with the HRVL agent were found to be infected with this or a related agent. Three of the 14 infected parents developed gastrointestinal symptoms at or around the time of their child's illness. The finding of HRVL infection in parents of HRVL infected children suggests a possible mode of transmission of this agent to the very young infant who is often not in contact with other infants and young children.

D. Inoculation of Volunteers with the HRVL Agent: A safety tested inoculum consisting of 1 ml (diluted in 30 ml water) of a 1:10 dilution of an approximate 2% filtrate prepared from a stool obtained from a 13 month old child (D) hospitalized with gastroenteritis has now been administered orally to 10 volunteers. (Each volunteer also received sodium bicarbonate shortly prior to administration of the virus containing solution). Six volunteers (2 of whom were reported on in last year's annual report) possessed relatively high or moderate levels of serum antibody to the HRVL and/or a substitute antigen, and none developed definite illness. However, two of the 6 developed a serologic response to the HRVL or a related agent. Virus was not detected in the stools of any of these 6 volunteers by IEM. Four volunteers with quite low antibody titers to the HRVL and/or related antigens were also studied in a similar manner. Two did not develop definite illness but one of the 2 not only shed a small quantity of HRVL agent in stool 4 days after challenge but also developed a serologic response to a HRVL related antigen; the virus negative volunteer did not develop a seroresponse. The two others with quite low antibody titers developed clinical signs and symptoms of illness as described below. On the day after challenge one developed chilly sensations, cramps, anorexia, fever (102°) with rapid recovery. He did not develop diarrhea and HRVL particles were not detected in stools; however, he did develop a serologic response to a HRVL related antigen. The other volunteer developed a definite gastroenteritis 2 days after challenge manifested by anorexia, diarrhea, and fever (101^4) with a slower recovery. The diarrhea lasted only one day; however, the volunteer continued to have systemic signs and symptoms for a few days and he shed the HRVL agent from day 2 to day 7 after challenge and also developed a serologic response to a HRVL related antigen. Thus, 2 of 4 volunteers with quite low antibody titers (CF vs HRVL and/or related antigens, neutralization vs NCDV, IF vs HRVL agent) developed clinical manifestations with one developing a frank gastroenteritis. In total, 5 of 10 volunteers challenged appear to have been infected with the HRVL agent. We have found from these studies that it is very difficult if not impossible, to find adults without pre-existing antibody to the HRVL or related agents. However, the fact that illness can be induced in some adults with little antibody is encouraging for future studies since the effectiveness of potential vaccines may thus be able to be evaluated in adult volunteers. Additional studies will be carried out to establish the illness rate induced by the HRVL agent in volunteers with little, if any, antibody.

E. Etiology of Gastroenteritis in Rural Bangladesh: We collaborated with Dr. R. Ryder of CDC (at his invitation) in a study attempting to elucidate the etiology of diarrhea in patients hospitalized in rural Bangladesh. Forty-eight patients admitted to a rural Bangladesh hospital with dehydration

secondary to diarrhea were examined for infection with the HRVL agent or enterotoxigenic Escherichia coli (ETEC). The diagnosis of HRVL infection was established by EM of stool filtrates and/or by a 4-fold or greater rise in NCDV CF antibody. Evidence of infection by heat-labile toxin-producing ETEC was obtained by stool culture and the adrenal-cell tissue culture system, whereas, evidence of infection by heat-stable toxin producing ETEC was obtained by stool cultures and the infant mouse test. Twelve patients, all less than 2 years of age, had evidence of HRVL infection, accounting for illness in 55% of the 22 patients who were less than 2 years old. None of these 22 children had evidence of ETEC infection. HRVL associated diarrhea lasted 5 to 6 days, often led to serious dehydration and was associated with vomiting and fever. Eleven cases of ETEC diarrhea were detected, accounting for 56% of the cases of diarrhea in the 18 patients who were over 10 years of age. Diarrhea associated with ETEC was sudden in onset, shorter in duration and caused pronounced dehydration. In a community survey, ETEC was isolated with equal frequency in the stools of control and case family members. The data suggest that in Bangladesh the HRVL agent is a common cause of diarrhea in infants and young children, whereas ETEC is a common cause of diarrhea in older children and adults.

F. Cell Culture Studies with the HRVL or Related Agents: Last year we reported the isolation and ten successful passages of a strain ("Fh") of HRVL agent in HEK cell culture. We have now carried this virus strain for a total of 16 serial passages. In higher passages small numbers of virus-like particles have been visualized in negatively stained preparations using EM. It is noteworthy the these particles have all been incomplete or subviral particles, which lack the outer capsid. Also at the 13th passage level ultrathin sections of infected cells were examined by electron microscopy, and reovirus-like particles were seen in vesicles bounded by rough endoplasmic reticulum. In high passage levels, which were also examined using indirect immunofluorescence, small numbers (usually less than 1%) of cells have been observed to contain viral antigens. To show that the fluorescent stainable viral antigen in HEK cultures after 13 serial passages was associated with the reovirus-like agent, we tested acute and convalescent sera from two patients who had gastroenteritis associated with the reovirus-like agent. Both patients developed antibody to the antigen in cell culture. Also hyperimmune guinea pig serum prepared against concentrated and purified human reovirus-like agent produced fluorescence staining while the pre-serum from the same animal did not.

We believe that these experiments indicate the cultivation of the human reovirus-like agent in human embryonic kidney cell cultures. However, since we have worked with other viruses which are currently indistinguishable from the human virus serologically, the possibility of a laboratory contaminant has been considered. However, 3 of the related viruses, the NCDV, the SA-11 and the "O" agent, are adapted to cell culture and produce cytopathic effects in bovine embryonic kidney and/or African green monkey kidney cultures; this newly isolated strain of virus does not. Currently we are investigating the possibility of stimulating the virus to grow more effectively in cell culture. The cell-culture adapted NCDV has also been studied extensively

in attempts to define better the growth characteristics for this group of RVL agents with the hope that such information could then be applied to the HRVL agent. It has been demonstrated that the use of serum-free medium is important, and that concentration of viral inoculum increases the number of infected cells. With these methods, the NCDV has been isolated from field cases of calf diarrhea; also the NCDV and the HRVL agent have recently been shown to replicate in monolayer cultures prepared from bovine embryonic intestine.

G. Animal Studies with the HRVL Agent: The newborn colostrum-deprived rhesus monkey was shown previously to be susceptible to infection and illness due to the HRVL agent; in those studies, five of seven newborn monkeys developed diarrhea 2-5 days after inoculation, and RVL particles were found in their stools. A total of 5 serial passages of the "D" strain of the HRVL agent has now been completed, and no illness has occurred except after the first passage. Infection on passages 1-4 was demonstrated on the basis of virus particle shedding in stools. The absence of diarrhea on the second and higher monkey passages of the HRVL agent suggests the possibility of rapid attenuation in the nonhuman primate. Since a goal in our project is the detection or development of a virus strain capable of infecting without producing illness, these observations will be studied further. Four recently-weaned (approx. 6 mos. old) rhesus monkeys maintained in isolation were studied by inoculating the same "D" strain of HRVL by stomach tube and by intraduodenal injection into each of two animals; no illness resulted, but at least one animal was infected, based on particle shedding in stools and serum antibody response. Two additional recently-weaned animals were fed partially purified HRVL agent ("D" strain) and neither developed illness, but one did become infected. Four newborn colostrum-deprived infant monkeys received 8th or 13th cell culture passaged HRVL agent; no illness developed. Finally, young patas monkeys (3-4.4 kg.) did not develop diarrhea which could be associated with the HRVL agent following inoculation via stomach tube.

The tissues from two caesarean-derived newborn monkeys inoculated with the HRVL agent and sacrificed 2 days later were studied further. At the time they were sacrificed, one monkey had diarrhea and one did not. Fluorescent stainable viral antigens were detected in mid-to distal small intestine of the ill monkey and only in distal small intestine of the well monkey. Fluorescent antigens were seen as cytoplasmic granules located primarily in intestinal lamina propria cells but were also observed in a mesenteric lymph node of the ill monkey. By EM, virus was also located mainly in subepithelial cells of intestinal tissues. It was seen in large numbers in cytoplasmic vesicles which also contained tubular structures suggesting these to be sites of virus replication.

Previously, we described studies in which two gnotobiotic colostrum-deprived newborn calves were shown to develop diarrhea following intraduodenal administration of second calf-passaged HRVL agent. Calves which were administered the HRVL agent in the form of human stool filtrate, did not develop diarrhea, but were infected based on seroresponse using IF and on the detection of RVL particles in stools. During the past year, virus was

serially passaged 3 additional times in newborn calves for a total of 5 serial passages. At each subsequent passage level diarrhea was observed. Five gnotobiotic calves were then inoculated at 3-5 days of age, and none developed diarrheal illness. Since it is therefore not possible to test for the development of resistance to disease because of the limited age range of susceptibility, we are now attempting to infect calves in utero and following this with a second challenge at birth. Such studies may help to define the degree of antigenic relatedness of human calf reovirus-like agent and may be important in future studies of immunoprophylaxis.

Intestinal lesions induced by third and fourth calf passaged reovirus-like agent of human infantile gastroenteritis were studied in gnotobiotic calves using light and immunofluorescent microscopy, and scanning and transmission electron microscopy. Calves were sacrificed and examined 0.5 hr, 3 hr, 7 hr, and 48 hr after the onset of diarrhea. The sequence of events in the small intestine was: (1) infection of the absorptive villous epithelial cells; (2) replacement of the tall columnar villous epithelial cells with cuboidal and squamous cells, shortening of the villi, enlargement of reticular cells and lymphocytic infiltrations into the villous lamina propria; and (3) repair. In calves studied at 0.5 hr and 3 hr following onset of diarrhea, fluorescent stainable viral antigens were detected in the mucosa of distal small intestine and a mesenteric lymph node. Frozen sections of the distal small intestine from one of these calves provided a valuable source of viral antigen for numerous indirect immunofluorescent antibody titrations of human and animal sera.

Finally, HRVL agent and NCDV-derived from calf stool have been used as a source of antigen for purification and initial characterization of viral nucleic acid and proteins (described later). This approach was made necessary because of the limited growth of the HRVL agent in cell culture.

Further studies were undertaken in gnotobiotic newborn piglets which were demonstrated previously to be susceptible to the HRVL agent with the development of a generally self-limited diarrheal illness. A total of 25 of 27 newborn piglets inoculated orally with the "Fh" strain of the HRVL agent have developed diarrhea; in these studies this agent was serially passaged 5 times. The "D" strain of the same virus was also shown previously to induce diarrhea in piglets. Virus shedding patterns of eight HRVL agent-infected piglets were completed this year. Fecal viral counts were highest before or at the onset of diarrhea. In diarrheic piglets, viral particles were observed for only 1-2 days after the onset of diarrhea. One infected piglet shed virus for four days but did not develop diarrhea. The presence of virus at or about the time of illness further supports the induction of diarrhea in piglets inoculated with the human reoviruslike agent.

Thus far we have not been able to induce diarrheal illness in older gnotobiotic piglets. None of seven animals challenged at 3 to 5 weeks of age with virulent HRVL agent developed diarrhea. Four of the 7 have, however, developed serum antibody detected by IF.

H. Biophysical Studies with the HRVL and Related Agents: The availability from experimental animal studies of a large quantity of calf stool containing the human reovirus-like (HRVL) agent provided an opportunity to perform biophysical and biochemical studies with this agent and thus enabled a comparison with related reovirus-like agents. The HRVL agent and NCDV (Cody strain) were extracted from diarrheal calf stools by treatment with Genetron (trifluorotrichlorethane) and purified by velocity centrifugation on 20-40% sucrose density gradients, followed by equilibrium centrifugation in cesium chloride density gradients (1.1 g/ml to 1.6 g/ml). Fractions with densities ranging between 1.38 g/cm^3 and 1.47 g/cm^3 were examined by electron microscopy and found to be free of contaminating, particulate debris and to contain large numbers of reovirus-like particles.

Cell culture grown SA-11 and "O" agent (in AGMK cells), and NCDV (in BEK cells) were also purified for comparative purposes during these studies. The virus in cell culture fluid was extracted with Genetron, and subjected to equilibrum centrifugation in cesium chloride density gradients followed by velocity centrifugation in 20-40% sucrose density gradients.

The RNA of Reovirus-like Agents: RNA was extracted from purified virus by treatment with phenol-chloroform (1:1) and precipitated in ethanol. By polyacrylamide gel electrophoresis of RNA it was possible to distinguish between the human and calf reovirus-like agents associated with diarrhea. These two viruses were seen to differ in as many as 5 of their RNA genome segments on the basis of electrophoretic mobility on 9% gels. In addition, comparison of the RNA segments of two other related viruses (the "O" agent and SA-11 virus) indicated that these viruses differed from the human and calf viruses as well as from each other. Evidence that all four viruses contain 11 double standard RNA segments was also suggested from electrophoretic patterns. Based on contour length measurement, the RNA segments of the human and calf viruses were divided into 4 size classes in contrast to the 3 size classes seen with reovirus type 2.

Transcriptase Activity in Reovirus-like Agents: It is known that reoviruses and orbiviruses contain an active transcriptase, capable of synthesizing messenger RNA (mRNA) in an in vitro system. A preliminary experiment was carried out using reovirus and orbivirus methodology to assay for transcriptase activity in NCDV core particles. NCDV purified from 25X concentrated bovine embryonic kidney cell cultures which contained primarily virus cores (particles with single capsids) was used as a source of transcriptase. Incorporation of tritium labeled uridine was used as an assay for detection of newly formed mRNA during the reaction. Trichloroacetic acid precipitated RNA was counted at 0, 30, 60 and 120 minutes after the additional of NCDV to the reaction mixture. A linear relationship was seen when the number of radioactive counts was plotted against time, indicating the presence of active transcriptase in the NCDV core particles. This technique is a useful way to make radioactively labeled mRNA and provides an opportunity to perform experiments which are more sensitive than electrophoresis experiments in detecting differences in RNA segments from the various reovirus-like agents. More specifically, hybridization and heteroduplex experiments using denatured genome RNA and radioactively labeled RNA are now possible.

Polypeptide Components of Reovirus-like Agents: Studies were initiated to determine the polypeptide composition of reovirus-like agents. The relative optical densities of purified HRVL agent and NCDV preparations at 260 um (for nucleic acid content) and 280 um (for protein content), Lowry test for total protein concentration, and dry weight determination were used as criteria for establishing purity prior to polypeptide analysis by electrophoresis. Purified HRVL agent and NCDV were solubilized in sodium dodecylsulfate (SDS) and mercaptoethanol and applied to SDS-polyacrylamide gels for analysis of polypeptides by electrophoresis. Data from preliminary experiments indicate that the virions of HRVL agent and NCDV contain 7 or 8 polypeptides which were visualized on the gels following Coomaissie blue staining. This information is important for correlating the number of polypeptides present in complete virions (i.e., those with the normal double capsid) with RNA segments which code for them. Following chemical or physical degradation of virus particles, this methodology will be used to determine which polypeptides are contained in the outer and inner capsids of virus particles. Such methodology may be important for the development of more sophisticated serologic methods such as radioimmunoassay.

Significance to Biomedical Research and the Program of the Institute

Acute infectious nonbacterial gastroenteritis is a common infectious disease which affects a broad segments of the population and was the second most common disease observed in a 10 year family study. Ultimate goals of prevention and therapy have been furthered by (1) the detection and preliminary characterization of etiologic agents of this disease, (2) the development of serologic assay systems by which the epidemiologic importance of the disease can be determined, (3) the probable in vitro cultivation of the HRVL agent, (4) the ability to induce illness in human volunteers and experimental animals administered the HRVL agent and (5) the ability to differentiate the HRVL agent from other related agents by biophysical methods. The progress in studying the role of the reovirus-like agent in the etiology of gastroenteritis in infants and young children represents a significant step in understanding this disease of world-wide importance.

Propose Course of Project

Intensive study of etiologic agent(s) from outbreaks of acute, infectious nonbacterial gastroenteritis and from clinical specimens from Children's Hospital will continue. In vitro propagation of the agent(s) will be studied further using a variety of cell culture systems and techniques. Further biophysical purification and characterization of these agents, development of more sensitive serologic assay systems, and the methods for rapid diagnosis of this disease will be major goals. Studies of the pathogenesis of disease in humans and in animal models will also be pursued. Development of a vaccine to prevent disease due to the human reovirus-like agent will be a major goal of this project.

Publications

Kapikian, A.Z., Feinstone, S.M., Purcell, R.H., Wyatt, R.G., Thornhill, T.S., Kalica, A.R. & Chanock, R.M.: Detection and Identification by Immune Electron Microscopy of Fastidious Agents Associated with Respiratory Illness, Acute Nonbacterial Gastroenteritis, and Hepatitis A. Perspectives in Virology IX, Chapter 2, pp. 9-47, 1975.

Thornhill, T.S., Kalica, A.R., Wyatt, R.G., Kapikian, A.Z., & Chanock, R.M.: Pattern of Shedding of the Norwalk Particle in Stools During Experimentally Induced Gastroenteritis in Volunteers as Determined by Immune Electron Microscopy. J. Infect. Dis. 132: 28-34, 1975.

Kapikian, A.Z.: Contributor to Control of Communicable Diseases in Man. Twelfth Edition, Ed. A.S. Benenson, M.D. Published by APHA 1975.

Dolin, R., Levy, A.G., Wyatt, R.G., Thornhill, T.S. & Gardner, J.D.: Viral Gastroenteritis Induced by the Hawaii Agent: Jejunal Histopathology and Seroresponse. Am. J. Med. 59: 761-768, 1975.

Torres-Medina, A., Wyatt, R.G., Mebus, C.A., Underdahl, N.R., and Kapikian, A.Z.: Diarrhea in Gnotobiotic Piglets Caused by the Human Reovirus-like Agent of Infantile Gastroenteritis. J. Inf. Dis. 133: 22-27, 1976.

Wyatt, R.G., Sly, D.L., London, W.T., Palmer, A.E., Kalica, A.R., VanKirk, D.H., Chánock, R.M., and Kapikian, A.Z.: Induction of Diarrhea in Colostrum-Deprived Newborn Rhesus Monkeys with the Human Reovirus-like Agent of Infantile Gastroenteritis. Arch. Virol. 50: 17-27, 1976.

Kapikian, A.Z., Kalica, A.R., Shih, J.W., Cline, W.L., Thornhill, T.S., Wyatt, R.G., Chanock, R.M., Kim, H.W., and Gerin, J.L.: Buoyant Density in Cesium Chloride of the Human Reovirus-like Agent of Infantile Gastro-enteritis by Ultracentrifugation, Electron Microscopy, and Complement-Fixation. Virol. 70: 564-569, 1976.

Ryder, R.W., Sack, D.A., Kapikian, A.Z., McLaughlin, J.C., Chakraborty, J., & Mizanur Rahman ASM.: Enterotoxigenic Escherichia coli and Reovirus-like Agent in Rural Bangladesh. Lancet 1: 659-662, 1976.

Kapikian, A.Z., Kim, H.W., Wyatt, R.G., Cline, W.L., Arrobio, J.O., Brandt, C.D., Rodriquez, W.J., Sack, D.A., Chanock, R.M. & Parrott, R.H.: Human Reovirus-like Agent as the Major Pathogen Associated with "Winter" Gastro-enteritis in Hospitalized Infants and Young Children. N. Engl. J. Med. 294: 965-972, 1976.

Metson, M.H., Morris, G.K., Sack, D.A., Wells, J.G., Feeley, J.C., Sack, R.B., Creech, W.B., Kapikian, A.Z., & Gangarosa, E.J.: Travelers' Diarrhea in Mexico: A Prospective Study of American Physicians and Family Members Attending a Congress. N. Engl. J. Med. (in press).

Karlowski, T.R., Chalmers, T.C., Lewis, T.L., Kapikian, A.Z., Lynch, J.M., Schaffer, G.W., & George, D.A.: A controlled Clinical Trial of Ascorbic Acid for the Common Cold. N.Y. Acad. Sci. 2nd Conference on Vitamin C, 258: 505-512, 1975.

Tyrrell, D.A.J., Almeida, J.D., Cunningham, C.H., Dowdle, W.R., Hofstad, M.S., McIntosh, K., Tajima, M., Zakstelskaya, L.Y., Easterday, B.C., Kapikian, A.Z., & Bingham, R.W.: Coronaviridae, Intervirology 5: 76-82, 1975.

Kapikian, A.Z.: The Coronaviruses in "Strategy for Chemoprophylaxis of Respiratory Virus Infections" CRC Press (The Chemical Rubber Company, Cleveland) (in press).

Sever, J.L., Kapikian, A.Z., Feinstone, S., Purcell, R., Gilkerson, M.R.: Hepatitis A and Down's Syndrome: Lack of an Association. J. Infect. Dis. (in press).

Kapikian, A.Z., Cline, W.L., Kim, H.W., Kalica, A.R., Wyatt, R.G., VanKirk, D.H., Chanock, R.M., James, H.D., Jr., & Vaughn, A.L.: Antigenic Relationships Among Five Reovirus-Like (RVL) Agents by Complement-Fixation (CF) and Development of New Substitute CF Antigens for the Human RVL Agent of Infantile Gastroenteritis. Proc. Soc. Exp. Biol. Med. (in press).

Kalica, A.R., Garon, C.F., Wyatt, R.G., Mebus, C.A., VanKirk, D.H., Chanock, R.M., & Kapikian, A.Z.: Differentiation of Human and Calf Reovirus-Like Antigens Associated with Diarrhea Using Polyacrylamine Gel Electrophoresis of RNA. Virology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00022-18 LID

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Study of Nonbacterial Respiratory Diseases in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Robert M. Chanock	LID/NIAID	Chief, LID/NIAID
Other Investigations:			
	Dr. Robert Belshe	LID/NIAID	Research Associate
	Dr. Linda S. Richardson	LID/NIAID	Staff Fellow

COOPERATING UNITS (if any)

Children's Hospital National Medical Center, Washington, D.C.

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9/12	3/12	6/12

SUMMARY OF WORK (200 words or less - underline keywords)

Previously an inactivated RSV vaccine was observed to induce an altered state of reactivity in young vaccinees so that they responded to their first infection with the virus by developing serious lower respiratory tract disease with an unusually high frequency. The possibility that live attenuated RSV vaccines might also induce an undesirable state of altered reactivity to disease has been raised on a number of occasions. Fortunately, experience during the past few years from a collaborative study with Drs. Parrott and Kim of Children's Hospital of D.C. does not bear out this fear. There was no evidence that 2 live attenuated RSV vaccine strains induced a state of altered reactivity to infection.

Project Description

Objectives: To delineate the epidemiology and pathogenesis of virus which cause respiratory disease in early life and develop methods for control of such illness.

Response of Live Virus RSV Vaccinees to Natural Infection with the Virus: Failure to Detect a Vaccine Associated Potentiation Effect.

Previously an inactivated RSV vaccine was observed to induce an altered state of reactivity in young vaccinees so that they responded to their first infection with the virus by developing serious lower respiratory tract disease with an unusually high frequency. The inactivated vaccine did not protect against RSV infection; 65% of the vaccinees became infected following exposure to the virus and this frequency did not differ from that of the control group. However, 80% of the RS vaccinees who were infected developed respiratory disease severe enough to necessitate admission to the hospital. This response was interpreted as a potentiation of disease induced by inactivated vaccine, probably through stimulation of an unbalanced immunological response in which systemic, but not local respiratory tract immunity was induced.

The possibility that live attenuated RSV vaccines might also induce an undesirable state of altered reactivity to disease has been raised on a number of occasions. Fortunately, experience during the past few years from a collaborative study with Drs. Parrott and Kim of Children's Hospital of D.C. does not bear out this fear. A total of 13 initially serum antibody negative infants received either the live 26°C adapted RSV vaccine (3 infants) or the live ts-1 mutant (10 infants) and these vaccinees were followed for their response to naturally occurring RSV infection during subsequent annual epidemics produced by the virus. Eleven of the 13 infants (3 in the 26°C adapted virus group and 8 in the ts-1 group) were infected with natural virus while under surveillance. Two vaccinees had 2 RSV infections under observation and thus there was a total of 13 infections detected. Twelve of the infections were silent, i.e., they were not associated with signs or symptoms of respiratory tract disease or fever. The one child who became ill in association with RSV infection had an upper respiratory tract illness and a maximum fever of 100^{6}. Thus there was no evidence that the 2 live attenuated RSV vaccine strains induced a state of altered reactivity to infection. On the contrary, the data suggest that the vaccines were protective since primary RSV infection is associated with febrile respiratory tract illness in 50% or more of instances.

Publications

Chanock, R.M.: Vaccines for non-bacterial disease of the lower respiratory tract. In Kirkpatrick and Reynolds (eds.) Immunologic Reactions of the Lung, Natl' Heart and Lung Institute Monograph, Marcel Dekker, Inc., New York, 1976 pgs. 375-405.

Chanock, R.M.: Respiratory syncytial virus epidemiology, in A.S. Evans, Viral Infections of Man: Epidemiology and Control, in press, 1975.

Chanock, R.M., Richman, D.D., Murphy, B.R., Spring, S.B., Schnitzer, T.S., and Richardson, L.S.: Current approaches to viral immunoprophylaxis. In Viral Immunology and Immunopathology, A.L. Notkins (ed.), Academic Press, 1975, pp. 291-316.

Parrott, R.H., Kim, H.W., Brandt, C.D., and Chanock, R.M.: Respiratory syncytial virus in infants and children. Prevent. Medicine 3: 473-480, 1974.

Kim, H.W., Leikin, S.L., Arrobio, J., Brandt, C.D., Chanock, R.M., and Parrott, R.H.: Cell-mediated immunity to respiratory syncytial virus induced by inactivated vaccine or by infection. Ped. Res. 10: 75-78, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00024-16 LID

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Laboratory Studies of Myxoviruses, Paramyxoviruses and Respiratory Syncytial Virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
168/12	60/12	108/12

SUMMARY OF WORK (200 words or less - underline keywords)

Influenza A and respiratory syncytial virus ts mutants were evaluated in vitro and in experimental animals for evidence of genetic stability and antigenicity. A double lesion ts recombinant of influenza A virus was produced which contained 2 ts lesions, each of which was relatively stable as regards reversion to wild type properties.

The further mutagenized ts-1 mutant of RS virus into which an additional lesion was induced with nitrosoguanidine was evaluated for genetic stability and was found to be significantly more stable than its ts-1 parent virus.

PHS-6040

Project Description

Objectives: (1) To produce conditional lethal temperature-sensitive (ts) mutants of myxoviruses, paramyxoviruses and RS virus for possible use in live attenuated vaccines; (2) to study the genetic basis for attenuation shown by temperature sensitive mutants; (3) to define the relative importance of the various host defense mechanisms in resistance to myxovirus and paramyxovirus infection and disease.

Major Findings

(A) Respiratory Syncytial Virus

Efforts in the RS program this year have been directed toward the following; (1) to define more precisely the genetic makeup of the RS virus genome through grouping of ts viruses into appropriate complementation groups; (2) to further study the genetic stability of the ts mutants in an effort to define the conditions under which a virus is most genetically unstable and therefore develop new criteria for the evaluation of ts mutants as potential candidates for use in immunoprophylaxis of RS virus disease in infants; (3) to further examine the phenotypic expression of the ts viruses; (4) to study the growth and pathogenesis of RS virus in animal models.

(1) Further characterization of the genetic composition of RS virus: In this laboratory ts mutants were grouped previously into 3 complementation groups based on their ability to complement one another at the restrictive temperature of 38° or 39°C. Group A consists of ts-1, ts-3, ts-4, ts-5, and ts-6. Groups B and C consist of single representatives, ts-2 and ts-7 respectively. In addition ts-2 is a plaque morphology mutant, since it forms atypical plaques which lack syncytia.

Based on the probable molecular weight of the RS virus genome, the existence of at least 6-7 complementation groups is likely. We therefore attempted to group other existing ts mutants into complementation groups. Five new ts mutants produced at Flow Labs in earlier years, ts-8, ts-9, ts-10, ts-11 and ts-12, were tested in our complementation test. Ts-9, ts-10, and ts-11 are clearly group A mutants since they do not complement with ts-1 but do with ts-2 and ts-7. Ts-9 and ts-12 are presently being characterized. Studies have recently begun on another existing ts mutant, a cold adapted RS A_2 virus (CA) produced at Flow Laboratories by serial passage of RS A_2 at 26°C in WI38 cells; its complementation group is not yet known. Further attenuated mutants of ts-1 (ts-1 NTG 1 and ts-1 NTG 16) derived by mutagenesis of ts-1 with nitrosoguanidine (NTG) retain their ability to complement with group B and C mutants but not group A indicating the additional lesion or lesions are not in the region of the complementation group B or C defect.

Since new complementation groups would aid in the genetic analysis of RS virus and highly attenuated mutants are desired as possible vaccine candidates, we have continued to isolate new ts mutants. A search for spontaneously occurring ts-mutants of RS revealed none in 265 clones of a 32°C grown suspension nor were any ts viruses detected in 435 clones of 40°C grown RS A_2 virus. UV light mutagenization of RS A_2 is currently being evaluated for production of new ts mutants. Another approach involves serial passage ts-1 at low temperature in hopes of introducing additional lesions into the genome as has previously occurred with other viruses. Ts-1 grows to a titer of 10^5 pfu per ml in BEK cells at 24°C by day 14 post-infection. Therefore 24°C was chosen as the temperature to passage ts-1. Ts-1 has thus far been passaged 10 times at 24°C. 10 clones picked from passage 5 and uncloned passage 10 of the virus showed no difference in efficiency of plaquing (EOP) as compared to the parent ts-1. Therefore additional passages of the virus at 24°C will be made. Similar experiments have been carried out with ts-2, and after 4 passages at 24°C no alteration in EOP was detected in any of 10 selected clones.

(2) Further study of the genetic stability of the ts mutants of RS: Previous studies with ts-1 showed it to revert to ts^+ virus in vitro (when grown at restrictive temperatures) and in vivo as determined by its ability to produce plaques at 38°. It is not known whether revertant ts-1 was responsible for the mild symptoms seen in young children who received the live ts-1 vaccine, however we believe any live RS vaccine should be genetically stable in vivo and in vitro. To assess the level of relative genetic stability among our ts mutants, a member of each complementation group and the NTG derived mutants were serially passaged three times in HEp-2 cells at 37°, 38°, or 39°C, and then assayed for revertant virus at 38°C. At 37°C and 38°C ts-1 was the least stable genetically, ts-7 was intermediate in stability, and ts-2 was the most stable of the original ts mutants. At 39°C, a temperature at which ts-1 does not produce infectious virus, ts-1 was the most stable. No revertants were detected at any passage level with this virus at 39°. Other group A mutants produced revertant virus at 39° depending on their level of growth at this temperature. Ts-5, the leaky group A mutant produced revertant virus in each of 10 tube cultures after three passages, ts-4 in 3 of 10, ts-3 in 1 of 10 and ts-6 in none of ten tubes. Ts-2 and ts-7 regularly produced revertant virus at 39° after three passages. Thus at subrestrictive temperatures the group A lesion is the most unstable, but at temperatures at which growth cannot be demonstrated ts-1 is genetically stable. CA virus reverts at about the same frequency as ts-1 at 37°C and 38°C but does not grow or revert at 39°C.

Passage of ts-1 NTG-1 and ts-1 NTG-16 at 37° or 38° and scoring for revertant virus indicate that ts-1 NTG-1 is considerably more stable than the parent ts-1, since revertants were not detected in 37° grown virus, and growth did not occur at 38°. Revertants were detected in 37° and 38°C grown ts-1 NTG-16 although to a lesser degree than with ts-1 under the same conditions. We are currently extending the temperature range to 35°C and 36°C for serial passage of the ts mutants in an attempt to detect additional differences in reversion among the NTG mutants and their original ts parent. Based on the

results of the genetic stability experiments, ts-1 NTG-1 and ts-2 are the most promising vaccine candidates, and vaccine suspensions of each are being prepared for administration to adult volunteers.

Recent reports that pretreatment of cells with IdU increases yields of infectious virus in a variety of virus-cell systems prompted us to look at the effect of IdU pretreatment of cells on the replication of wild type RS and the ts-1 mutant. Pretreatment of cells 48 hours with concentrations of 50-200 ug/ml of IdU increased viral titers as much as 32 fold with both wild type RS and ts-1 at 32°C. In contrast when ts-1 was grown in IdU treated cells at restrictive temperature, an increase in viral titer was not noted; however revertant virus was detected earlier and more consistently in IdU treated cells. We therefore intend to screen all ts mutants at 35-39° in IdU treated cells as another test for genetic stability.

(3) Phenotypic characterization of ts lesions: Experiments were designed to establish whether the ts lesions of RS mutants are early or late in the replicative cycle of the virus. ts-1, ts-2, and ts-7 adsorb to HEp-2 cells with equal efficiency at 24°C or 40°C and do not differ from wild type virus. Thus the mutants do not have an adsorption defect. Temperature shift experiments were performed by holding virus infected tissue culture tubes for various times at 32°C before shifting to 39°C and then harvesting all tubes at 24 hours. Wild type RS A_2 virus infected cultures contained the same titer of virus for all of the shift times. Ts-1 required 13 hours at permissive temperature before shifting to restrictive temperature to produce infectious virus. Other group A ts mutants showed the same pattern of growth on temperature shift as ts-1 except ts-5 which was sufficiently leaky to preclude analysis. Thus the group A lesion appears to be expressed late in the replicative cycle. Ts-2 showed no difference at any of the shift times and was parallel in growth to wild type virus. Ts-7 required six hours at permissive temperature to produce infectious virus and thus appeared to have an early lesion.

Ts-2, the group B lesion representative, appears to be ts in plaque formation but not ts in growth. We postulate that ts-2 is deficient in biologically active fusion protein and this defect inhibits plaque formation, i.e. virus spread, of ts-2 at restrictive temperature but not growth at restrictive temperature.

Growth studies in tissue culture with the NTG mutants revealed the viruses to remain eclipsed for 24 hours at 32°C. A two day temperature shift experiment (32°→39°C) demonstrated that ts-1 NTG-1 required the longest period of time at permissive temperature, 21 hours, to produce infectious virus. Ts-1 NTG-16 required only 14 hours at permissive temperature to produce virus.

(4) Animal studies with RS virus and its ts mutants: Growth of wild type RS A_2 virus, ts-1, and ts-2 was studied in infant ferrets. In infant ferrets less than three days old wild type RS A_2 virus grew to a titer of 10^6 pfu of virus per gram of nasal turbinate and 10^5 - 10^6 pfu per gram of lung, confirming the work of Porter and Prince. Ts-1 was partially restricted in

growth in the lungs but not in the nasal turbinates. Ts-2 was reduced in growth in both the lungs and nasal turbinates indicating that the mutant was more defective than ts-1 in the infant ferret. With ts 2 there was a 4 day delay in attaining peak viral growth and the titer was 100 to 1000 fold less than with RS A_2 suggesting that ts-2 was defective in spreading in vivo.

Immunofluorescent studies on frozen sections of nasal turbinates and lungs from infected ferrets revealed that viral antigen was detected in 100% of cells lining the nasal turbinates. Antigen was not seen in the trachea, bronchi, or bronchioles. The lungs contained individual infected cells distributed throughout the lung parenchyma. Sections of formalin fixed lung and nasal turbinates failed to reveal definite pathology; occasional areas of denuded nasal epithelium were seen in infected infant ferrets but not in controls.

Studies of ts-1 NTG-1 in hamsters showed that limited growth of ts-1 NTG-1 occurred in the nasal turbinates, but not in the lungs. Growth was not detected in either location with ts-1 NTG-16. Ts-1 NTG-1 conferred significant protection against subsequent challenge with the wild type, although not as complete as following infection with ts-1 or wild type RS virus. Serological analysis of infected hamsters indicated that 7 of 8 developed an antibody response to ts-1 NTG-1, while only 3 of 8 responded to ts-1 NTG-16. This probably accounts for the lack of protection against wild type challenge in the ts-1 NTG-16 infected hamsters.

(B) Influenza Viruses

1. Isolation and characterization of temperature-sensitive mutants of influenza A virus: The purpose of further isolation and characterization of ts mutants of influenza A is to (1) derive a series of recombinants which have a single lesion or cluster of lesions on one of the functional portions of the influenza A virus genome with the aim of producing ts viruses with lesions specific for each of the viral genes; (2) derive separate mutants which have a ts defect in the same segment of the influenza A virus genome but which differ with regard to temperature sensitivity; (3) further evaluate ts viruses in seronegative volunteers to explore more fully the relationship between attenuation, site of genetic lesion and level of temperature sensitivity. Such single lesioned mutants could also serve as genetic probes for characterization of the lesion or lesions transferred from an older ts vaccine to a newly emerged influenza A virus strain possessing new surface glycoprotein antigens in order to verify that a putative ts recombinant virus possesses only the desired ts lesions of its attenuated parental strain.

Previously all ts mutants produced in this laboratory were produced by mutagenesis with 5-fluorourcail. Over the past year we have explored the use of additional mutagens including nitrosoguanidine (NTG), hydroxylamine (HA) and ultraviolet (UV) light. Over 600 clones of virus treated either directly with NTG or exposed to NTG during replication were examined for their level of temperature sensitivity. Only two clones were ts and these were further evaluated for efficiency of plaque formation (EOP) at various

temperature, locus of ts lesion, and replication and genetic stability in hamsters. The viruses behaved inconsistently in tissue culture—i.e., the EOP varied from 37° to 39° in different tests. Genetic analysis was therefore difficult, but evaluation of these viruses in hamsters demonstrated that their reversion rate was too high for the mutants to be useful candidate vaccine viruses. NTG therefore was not considered an efficient mutagen for influenza A virus. Similarly HA was evaluated and of 200 clones of virus treated with mutagen none were ts. In contrast, 5-FU was used again and 2 of 120 clones of mutagenized virus were ts. These are being further evaluated.

Since the H3N2 ts-1[E] recombinants exhibited reversion in doubly-seronegative children as described previously, it was necessary to obtain more stable ts mutants. If temperature-sensitivity of a mutant were the result of a deletion mutation, the mutant would not be expected to have a detectable back-mutation rate. We attempted to produce such mutants by ultra-violet (U.V.) irradiation of the Udorn/72 (H3N2) wild type virus. The virus was irradiated with U.V. resulting in a 1000-fold reduction in infectivity. Surviving virus was inoculated onto bovine kidney monolayer cultures. Plaques were picked after 4 days incubation at 33°C and each of the plaque populations was tested for ability to produce plaques at 39°C. Of a total of 2008 plaques tested, 9 clones showed distinct temperature-sensitivity. Efficiency of plaque formation of the 9 ts mutants was examined at 33°, 37°, 38°, 39° and 40°C. One had a shutoff temperature of 37°C; 3, 38°C; 4, 39°C; and 1, 40°C. 585 of the 2008 plaque populations were tested for temperature-sensitivity using three kinds of cells, i.e., bovine kidney (BK), rhesus monkey kidney (RMK) and canine kidney (MDCK) cells. 5 clones were found to exhibit temperature-sensitivity only in MDCK, but not with BK or RMK. These mutants should be useful for analysis of the mechanism of host specificity.

2. Further characterization of ts-1[A] viruses: The Hong Kong/68-ts-1[A] virus was more restricted than the Hong Kong/68-ts-1[E] in hamsters and man and therefore was considered a candidate donor of its ts lesions to produce vaccine strains of new epidemic wild type viruses for use in doubly seronegative individuals.

The level of replication in nude mice of Udorn/72-ts-1[A] ts recombinants clone 189 and clone 46 was compared to that of the Hong Kong/68 and Udorn/72 ts-1[E] viruses and wild type virus. The ts-1[A] viruses were approximately 100-fold more restricted in growth in both the nasal turbinates and lungs than the ts-1[E] viruses. In addition the ts-1[A] clone 189 and clone 46 recombinants were more stable genetically than the ts-1[E] viruses. The level of replication and genetic stability were similar for the clone 189 virus (which possessed only the complementation group 1 lesion) and the clone 46 (which possessed both group 1 and 5 lesions). This observation suggested that the group 5 lesion contributed little to the behavior of the ts-1[A] recombinants in vivo. For this reason the Udorn/72 ts-1[A] clone 189 virus may be suitable as a donor of its ts lesion for production of new recombinant vaccine strains.

One important aspect of the ts-1[A] virus and its recombinants needs clarification. The reduction of replication of the recombinants in the nasal turbinates, the inability of the Hong Kong/68-ts-1[A] virus to infect seronegative adult volunteers and the low level of replication in ferret tracheal organ culture at 33°C suggest that the Hong Kong/68-ts-1[A] virus and its Udorn/72-clone 46 and 189 recombinants might also possess a non-ts lesion that restricts growth at permissive temperatures. Further genetic analysis of the Udorn/72-ts-1[A] clone 189 virus and its recombinants needs to be undertaken to determine if there is a non-ts lesion present in the ts-1[A] virus and to determine if it segregates independently of the complementation group 1 lesion.

3. Production of a ts virus possessing two ts lesions by recombination of two genetically distinct ts donor viruses: Studies with a recombinant produced by a mating the ts-304 virus (group 4 ts lesion) and ts-1[E] virus (group 1 and 2 ts lesions) indicated that it possessed both the group 4 and group 1 ts lesions. Evaluation in nude mice indicated that this virus was greatly restricted (over 1000-10,000 fold more suppressed than ts-1[E] virus) in both the upper and lower respiratory tract. In addition, this virus was mated with A A/Ga/101/74 wild type virus in an attempt to obtain a Ga/74 virus that possessed both the group 1 and 4 ts lesions. However, over 30 ts recombinants were analyzed and each possessed only the group 1 lesion. Therefore, this "double" ts recombinant was considered too attenuated and a poor donor of its ts lesions; thus other donors of doubles lesions were sought.

Another double ts mutant was produced by a mating of the Udorn/72-ts-1[A] clone 189 (stable group 1 lesion) and Hong Kong/68-315 mutant (group 5 lesion). These viruses were chosen because they each exhibited an approximate 10,000 fold restriction of replication in the lungs and because genetic analysis revealed that neither the group 1 nor the group 5 ts lesion was on the genes that coded for the hemagglutinin and neuraminidase surface glycoproteins. One of 113 ts recombinants produced by a mating of the 189 and 315 viruses possessed the group 1 and group 5 lesions. This double ts recombinant virus was more restricted in replication at 37° than either parent virus. The double ts recombinant failed to replicate in the lungs of hamsters, but readily replicated in the nasal turbinates, although at a reduced level compared to the wild type virus. In addition, this virus was recently used as a donor of its 2 ts lesions to the A/Victoria/3/75 wild type virus.

4. Further transfer of ts lesions by recombination: The current epidemic strain of influenza A/Vic/3/75 was mated with 3 donor ts viruses: (1) Hong Kong/68-ts-1[E] virus (group 1 and group 2 lesions); (2) Udorn/72-ts-1[A] clone 189 virus (group 1 lesion); and (3) Udorn/72-ts-IA2 virus (group 1 and group 5 lesions).

One-hundred twenty plaque progeny from the HK-ts-1[E] X Vic wild type cross were examined for ts phenotype. Twenty-three were ts for a recombination frequency of 18%. Of these 23, 6 possessed both surface antigens (i.e. hemagglutinin and neuraminidase glycoproteins) of the Victoria/75 wild type

parent. Of these 6, 2 possessed both ts lesions present in the Hong Kong/68-ts-1[E] donor. Two others possessed the group 1 lesion only, and the remaining two possessed the group 2 lesion. A virus suspension for administration to volunteers that contains 1 X 10^8 pfu/ml and contains both ts-1[E] lesions has been prepared and is being safety-tested for clinical trial. Evaluation of the level of replication of this recombinant in hamsters indicated that it resembled its ts parent. An interesting finding with the Victoria/75-ts-1[E] recombinants is that the plaque size of the recombinant clones in MDCK cells is dependent on the neuraminidase type of the virus, i.e., viruses with the Victoria neuraminidase form small plaques, whereas those with the Hong Kong-like neuraminidase form large plaques.

Thirteen ts recombinants of the Udorn/72-ts-1[A] clone 189 X Victoria/75 wild type that have both surface antigens of the Victoria/75 wild type virus have been identified and cloned. Extensive genetic and biological evaluation will be undertaken to determine if any of these ts recombinants possess a non-ts lesion in addition to the ts lesion. A suitable candidate will be selected for evaluation in adults and later in children.

Recombination of the Victoria wild type virus and a ts donor which possesses group 1 and group 5 ts lesions is complete. These ts recombinants are being characterized for antigenic and genetic properties.

5. Studies in the hamster with ts^+ viruses produced from ts mutants: ts^+ viruses (possessing the temperature-sensitivity phenotype of wild type virus) were derived by two methods--reversion of ts virus in the hamster lung and recombination between two genetically distinct ts viruses. When ts^+ revertants or ts^+ recombinants were administered to hamsters, the following observations were made: 1) the ts lesion can be the sole determinant of growth restriction in the lung and by inference, attenuation; 2) some ts viruses may possess non-ts lesions that also restrict replication of virus in the lung; 3) ts lesions produce a greater restriction of growth in the warmer lower respiratory tract (37°C) than in the cooler nasal turbinates, while non-ts lesions do not produce such a selective restriction effect. Finally, restriction of growth of ts viruses in the lower respiratory tract was attributable in most instances to the effect of the ts lesions.

Studies with ts^+ recombinants indicate that the Hong Kong/ts-1[E] donor virus does not possess a non-ts lesion and that the level of attenuation of this virus is specified by the ts-1[E] ts lesions. When non ts lesions were present they could be detected by the ts^+ recombinant method. This technique will be used to probe the genetic makeup of the ts-1[A] recombinants.

6. Production of ts mutants of an A/New Jersey/8/76 virus (Hswine N1): A swine-like virus (A/NJ/8/76) from Fort Dix, N.J. was isolated in avian-leucosis virus-free, specific pathogen-free eggs by Dr. Allan Kendal and sent to us in its first egg passage. The infectivity of this virus was comparable in MDCK tissue culture and eggs, but was 50-fold reduced in rhesus monkey kidney tissue and 1000-fold reduced in bovine kidney tissue.

Since recombination and cloning of our recombinants is performed in primary bovine (calf) kidney culture, the A/NJ/8/76 virus was adapted to grow in this substrate by 8 passages in BK tube cultures followed by plaque to plaque passage. Recombination of A/NJ/8/76 virus with the Hong Kong/68-ts-1[E] virus has been performed and ts recombinants have been identified. These recombinants are currently under study.

(7) Comparison of the genetic and biological properties of 5 FU induced mutants and cold adapted variants of influenza: We have been concerned with developing and characterizing a series of ts mutants to serve as master donors for vaccine strains. To this end we have derived a series of 5 FU induced ts mutants and characterized them with respect to complementation group (on the basis of shared lesions) and genetic stability (on the basis of reversion rate to the ts^+ phenotype on replication in the hamster lung and nasal turbinates). The 7 complementation groups derived from these mutants have been used subsequently to determine the genetic lesions in other mutants and recombinants.

Massaab, Kendal, Davenport and their collaboratories have taken another approach to selecting viruses with differential ability to replicate in the lower and upper respiratory tract. They have sought cold adapted variants, that is viruses that can replicate equally well at 25° and 33°C; wild type influenza virus strains do not replicate efficiently at 25°C but can be adapted to grow at low temperature either by a stepwise process in which they are passaged at successively lower temperatures (differing by 3°C) or by blind passage at 25°C for several cycles followed by plaque selection at 25°C. We have begun preliminary biological and genetic characterization of a cold-adapted parent PI7 (derived from the A/AA/60/60-H2N2 virus), and 2 of its cold recombinants CR6 (Queensland/6/72-H3N2) and CR12 (A/AA/9/73 H3N2). The parental virus and the 2 recombinants were shown to be ts in addition to being cold adapted. A third cold recombinant CR13 (Dunedin/4/1972-H3N2) was not ts. All 3 ts cold variants were shown by genetic analysis to share the complementation group 1 lesion; they underwent genetic interaction with members of the other 6 complementation groups but not with a mutant which contained the group 1 ts lesion. The shutoff temperature of the 3 ts cold variants varied, PI7: 37°C; CR6: 37°C; and CR12: 38°C. In addition, these viruses were studied in the hamster animal model. As was characteristic of the 5 FU ts mutants and ts recombinants that we have previously studied, the cold variants were more restricted in their replication in the lungs than in the nasal turbinates and the viruses with the lower shutoff temperature (PI7 and CR6) were more restricted than the one with the higher shutoff temperature (CR12). We also studied the genetic stability of the viruses by examining isolates derived from organ homogenates for the presence of virus with the ts^+ (wild type) phenotype. Revertant virus was found only in one lung and 2 nasal turbinate isolates from animals infected with the CR12 virus. The level of reversion of the 2 37°C shutoff cold adapted viruses was lower than that found for ts-1[E] recombinants which have both the group 1 and group 2 lesions and a shutoff temperature of 38°C.

The above described preliminary analysis of the cold variants raised several questions: (i) why did the CR6 and CR12 recombinants have different shutoff temperatures despite being derived from a common ts parent; (ii) are the cold "recombinants" true recombinants or are they actually cold variants selected during the recombination procedure which was carried out at 25°C (a temperature restrictive for wild type virus); (iii) do all cold variants which are ts share the group 1 lesion; (iv) are cold variants more stable in hamsters than ts mutants with a similar level of replication in vivo and a similar shutoff temperature in vitro?

To answer these questions we examined 6 cold variants derived independently from a variety of wild type strains. These were: A/AA/Marton/ 43(H0N1), A/FM/1/47(H1N1), A/AA/2/65(H2N2), A/AA/2/67(H2N2), A/Aichi/2/68(H3N2) and A/AA/1/70(H3N2). We also studied 10 clones of virus (CR18) derived from recombination between the Scotland/840/74 strain and the P17 cold parent. The basic findings were as follows: (i) all of the Scotland clones (CR18) and 4 of the 6 independently derived cold variants were also ts (the 1943 and 1947 strains were not); (ii) all of the ts cold variants and recombinants shared the complementation group 1 lesion. Three of the cold variants also had additional lesions; the 1967 strain had group 3 and 5 lesions, the 1968 strain group 2 and 6 lesions and the 1970 strain group 3 and 6 lesions.; (iii) among the Scotland clones 6 had the neuraminidase subtype of the P17 parent and 4 had the subtype of the Scotland parent. This finding demonstrated that recombination had taken place rather than selection of cold variants at the recombination temperature (25°C); (iv) among the Scotland clones 4 had a shutoff temperature of 37°C and 6 had a shutoff of 38°C. This finding suggests that (a) factors other than the group 1 lesion play a role in the shutoff temperature or (b) that in the process of recombination there was some alteration of the ts lesion present on the group 1 gene. (v) Another hamster study was carried out to compare the restriction in growth and reversion rate of the parental P17 and 4 cold recombinants of P17 which included 2 Scotland 1974 clones, CR18 clone 6 and CR18 clone 7. The latter 2 viruses differed only in their shutoff temperature, 38° and 37°C, respectively. The findings were in accord with previous results in that the restriction of growth in the hamster lung correlated with the shutoff temperature, i.e., the lower the shutoff temperature—the greater the restriction. Cold adapted viruses were not inherently more stable genetically in vivo than ts recombinants with a similar level of temperature sensitivity. The CR 12 and CF18-6 viruses, with a shutoff temperature of 38°C, were more stable in vivo than the Hong Kong/68-ts-1[E] virus which also shutoff at 38°C. However, the Udorn/72-ts-189 recombinant was more stable (i.e., revertants were not detected) than the CR18-7 although both recombinants had a 37°C shutoff temperature for plaque formation.

(8) The nature of the cold adaptation lesion: The genetic findings suggest that the cold adaptation property might be linked in some manner to the group 1 lesion. In order to ascertain whether this was so we studied the ts and cold adaptation properties of 2 sets of viruses: (i) revertant viruses with the ts$^+$ phenotype isolated from the hamster studies and (ii) recombinant

viruses prepared at 34°C using the influenza A 1943 (HON1) Marton virus as one parent and the CR12 virus as the other. Among these virus populations we could identify viruses with various combinations of the 2 properties: ts recombinants which were cold adapted and some which were not; revertant ts$^+$ viruses which were no longer cold adapted and some which retained the cold adaptation property. Thus the question of the correlation of the ts and cold adaptation properties is at present not resolved. It seems likely that the group 1 lesion may code for a component of the viral RNA transcriptase complex. If this is so, it is possible to envision alterations in the structure of the protein such that its temperature optimum would make it functional or nonfunctional at various temperatures in the 25-39°C range. Further work is in progress to determine whether lesions in groups 2, 3, 5 and 6 segregated from the 1967 and 1968 cold adapted strains also carry with them cold adaptation as well as ts properties.

(9) The use of genetic analysis to characterize the biochemical nature of the ts lesions in the 7 complementation groups of 5 FU ts mutants of Hong Kong virus: Sugiura, Kilbourne, Ueda, Tobita, Krug and their collaborators have developed and characterized a series of 5 FU ts mutants of the WSN strain. These mutants have been divided into 7 complementation groups by genetic analysis and the biochemical defect of 6 of the groups has been established. We sought to determine the biochemical defect in members of each of our 7 complementation groups by carrying out a genetic comparison between the Hong Kong and WSN sets of 5 FU ts mutants. On the basis of this comparison we have established that HK 1 = WSN 1, C RNA defect; HK 6 =WSN 2, V RNA defect; HK 5 = WSN 3, C RNA defect. 3 of the WSN groups 4 (neuraminidase defect), 6 (hemagglutinin defect) and 7 (defect unknown) complement all 7 Hong Kong groups. Likewise 4 of the Hong Kong groups (2, 3, 4, 7) complement all 7 WSN groups. WSN group 5 complements with Hong Kong groups 1, 3, and 5, but does not appear to complement with the other 4 groups at a high level. If a correspondence can be demonstrated between the WSN group 5 and one of the Hong Kong groups then the total number of complementation groups accounted for by the 2 sets of mutants would be 9, a number which approximates the number of unique pieces of viral RNA demonstrated by polyacrylamide gel electrophoresis. The Hong Kong and WSN groups with unassigned function may correspond to genetic material coding for membrane protein, nucleocapsid or non-structural protein assuming that none of these proteins also has a role in V RNA or C RNA synthesis. The absence of Hong Kong mutants with ts lesions in the genes controlling the hemagglutinin and neuraminidase glycoproteins had been suggested by our previous findings using a marker rescue technique. It is possible that the cold variants also share some lesions with the WSN complementation groups other than WSN 1. Studies are currently underway to ascertain if this is true.

Publications

Richman, D.D., Murphy, B.R., Baron, S., and Uhlendorf, C.: Three strains of influenza A virus (H3N2): Interferon sensitivity in vitro and interferon production in volunteers. J. Clin. Microbiol. 3: 223-226, 1976.

Richman, D.D., Murphy, B.R., Cline, W.L., and Alling, D.W.: Determination of influenzavirus neuraminidase inhibition titres. Bull. World Health Organ. 52: 233-234, 1975.

Spring, S., Nusinoff, S., Tierney, E., Richman, D., Murphy, B., and Chanock, R.: Temperature-sensitive mutants of influenza virus. VIII. Genetic and biological characterization of ts mutants of influenza virus A (H3N2) and their assignment to complementation groups. Virology 66: 542-550, 1975.

Richman, D., Murphy, B., Spring, S., Coleman, M., and Chanock, R.: Temperature-sensitive mutants of influenza virus: IX. Genetic and biological characterization of ts-1[E] lesions when transferred to a 1972 (H3N2) influenza A virus. Virology 66: 551-562, 1975.

Murphy, B., Spring, S., Richman, D., Tierney, E., Kasel, J., and Chanock, R.: Temperature-sensitive mutants of influenza virus. VII. Transfer of the ts-1[E] lesions to a wild type influenza A virus with the H0N1 surface antigens. Virology 66: 533-541, 1975.

Schnitzer, T.J., Hodes, D.S., Gerin, J., Camargo, E., and Chanock, R.M.: Effect of 2-deoxy-D-glucose and glucosamine on the growth and functions of respiratory syncytial and parainfluenza 3 viruses. Virology 67: 306-309, 1975.

Spring, S., Nusinoff, S., Mills, J., Richman, D., Tierney, E., Murphy, B., and Chanock, R.M.: Temperature-sensitive mutants of influenza virus. VI. Transfer of ts lesions from the Asian subtype of influenza A virus (H2N2) to the Hong Kong subtype (H3N2). Virology 66: 522-532, 1975.

Murphy, B.R., Richman, D.D., Spring, S.B., and Chanock, R.M.: Use of temperature sensitive mutants of influenza A virus as live virus vaccine strains—Evaluation in laboratory animals, adults and children. Postgrad. Med. J., June 1976.

Schnitzer, T.S., Richardson, L.S., and Chanock, R.M.: Growth and genetic stability of the ts-1 mutant of respiratory syncytial virus at restrictive temperatures. J. Virol. 17: 431-438, 1976.

Murphy, B.R., Spring, S.B., and Chanock, R.M.: Blueprint for use of a live virus vaccine to prevent a pandemic of influenza A virus. In Proceedings of a Symposium of the Working Group on Pandemic Influenza, Switzerland, 1976, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00025-15 LID

PERIOD COVERED
July 1, 1976 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Mycoplasma pneumoniae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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30/12	12/12	18/12

SUMMARY OF WORK (200 words or less - underline keywords)

The influence of aerosol particle size and route of administration of M. pneumoniae on the course of experimental infection was studied in golden Syrian hamsters. In contrast to large-particle aerosols, which were deposited in the upper respiratory tract (URT), small-particle aerosols were distributed throughout the entire respiratory tract and were more effective in initiating infection and conferring protection following challenge. In other studies, hamsters inoculated orally with either wild-type or ts 640 M. pneumoniae underwent a silent URT infection and were significantly resistant to subsequent intranasal challenge. In an effort to establish an experimental disease with a closer resemblance to human disease 3 rhesus monkeys were inoculated via the URT with 10^7 CFU. Each animal underwent an asymptomatic infection with a serological response and shedding of the organism. Inoculation of 10^7 CFU in the URT and LRT of 3 squirrel monkeys also resulted in a silent infection. Subsequent challenge of these monkeys was followed by a shorter, more restricted infection without accompanying disease. There was no evidence of transmission of the organism to broth-inoculated cagemate controls.

Project Descrption

Objectives: (1) To study the effect of route of administration, site of colonization, dose and particle size of inoculum on the development of M. pneumoniae disease and subsequent immunity; (2) to develop a simian model for M. pneumoniae disease for use in studies of pathogenesis and immunity as well as for vaccine safety testing.

Progress

(1) Influence of Particle Size and Route of Administration of M. pneumoniae on the Course of Experimental Infection

In collaboration with USAMRIID, Frederick, Maryland, hamsters were exposed to wild type M. pneumoniae in a small particle (2.4 um) or large particle (10 um) aerosol. In addition, the organism was given by the intranasal route to anesthetized hamsters. The same dose of organisms was administered by aerosol or the intranasal route. Both types of aerosol preparations infected hamsters but there was a difference in distribution of infection. Large particles were deposited primarily in the upper respiratory tract and infection remained localized primarily to this area. In contrast, small particle aerosol was distributed throughout the respiratory tract and infection involved the whole tract. Small particle aerosol was more effective in initiating infection. In addition, small particle aerosol induced pulmonary infection was associated with the development of lung lesions which were strikingly similar to those caused by intranasal instillation of organisms to anesthetized hamsters. In contrast, large particle aerosol infection did not induce appreciable pulmonary pathology. Both types of aerosol infection induced significant resistance to pneumonia caused by intranasal instillation of virulent wild type M. pneumoniae, however, the small particle aerosol was more effective in this regard.

In other studies the intranasal and oral routes of infection were compared using virulent M. pneumoniae and an attenuated mutant, ts 640. Both organisms infected the entire respiratory tract when given intranasally but only virulent M. pneumoniae induced the development of pneumonia, whereas infection with ts 640 was silent. Nonetheless, both types of organisms stimulated significant resistance to subsequent intranasal challenge with virulent M. pneumoniae. When the virulent and ts 640 organisms were given to hamsters by the oral route infection was limited primarily to the upper respiratory tract and pneumonia did not develop or was mild. Orally infected hamsters, nevertheless, were significantly resistant to the development of pneumonia following subsequent intranasal challenge with virulent M. pneumoniae. Oral inoculation may provide a natural and convenient means of obviating residual virulence associated with the use of attenuated ts mutants of M. pneumoniae in man.

(2) Experimental Infections of Simian Species

Knowledge of the pathogenesis of pneumonia due to Mycoplasma pneumoniae has been derived primarily from experimental infection of rodents. As part of an effort to establish a model with a closer resemblance to man, three seronegative, young, adult rhesus monkeys (Macaca mulatta) were inoculated with M. pneumoniae ($10^{7.4}$ CFU per animal) by oropharyngeal administration of coarse-particle aerosol. Five to six days after exposure of the animals, cultures obtained from the upper respiratory tract were positive for M. pneumoniae. Each animal subsequently developed a serologic response, as determined by complement-fixation, complement-mediated killing, and tetrazolium-reduction inhibition techniques. Infection was subclinical, and serial chest roentgenograms failed to disclose pneumonia throughout the course of infection. Blood cell counts and titers of cold agglutinins remained unchanged. Although M. pneumoniae was recovered from the upper respiratory tract of two monkeys for 50 days, there was no evidence of transmission of infection to cage-mate controls inoculated with broth.

In collaboration with BOB, FDA each of three seronegative young adult male squirrel monkeys (Saimiri sciureus) were inoculated intratracheally by direct laryngoscopy with $10^{6.5}$ CFU of M. pneumoniae at 5 month intervals. Broth-inoculated control animals were housed with the infected monkeys. Seven to 14 days following the initial inoculation, each of the three squirrel monkeys which received M. pneumoniae became infected as determined by isolation of the organism from the upper respiratory tract. The duration of shedding ranged from 28 to 70 days in the infected animals. All infections were subclinical and no evidence of pulmonary disease was detectable by chest X-ray. Attempts to isolate M. pneumoniae from the broth-inoculated cagemate controls were uniformly unsuccessful. Reinfection of two squirrel monkeys 60 days after cessation of shedding resulted in a markedly shortened period of shedding (13 days) for one animal and failure to recover M. pneumoniae from the other animal. Serologic analysis of samples collected at weekly intervals is now in progress.

Significance of Program to the Institute

M. pneumoniae is an important cause of lower respiratory tract disease, especially during the first 2 decades. Attempts to develop attenuated mutants for use in immunoprophylaxis and to achieve a better understanding of the pathogenesis of disease are relevant to the Institute's mission of disease prevention and control.

Proposed Course of Study

The effect of site of deposition and size of inoculum on the course of M. pneumoniae infection and disease will be studied more extensively next year. Information gained from such studies may prove helpful in the development of techniques for administration of live attenuated M. pneumoniae mutants for the purpose of immunoprophylaxis. Use of a proper sized inoculum

delivered to the appropriate region of the respiratory tract may prove decisive in achieving the desired goal of an asymptomatic, immunizing infection.

In addition, the concept that M. pneumoniae pulmonary disease is caused in part by an immune response induced by prior subclinical infection will be studied using the simian model.

Publications

Greenberg, H., Prescott, B., Brunner, H., James, W., and Chanock, R.M.: Sharing of glycolipid antigenic determinants by Mycoplasma pneumoniae In Proceedings of the Symposium on New Approaches for Inducing Natural Immunity to Pyogenic Organisms, 151-156, 1974. DHEW Publication No. (NIH) 74-553.

Friedlaender, R.P., Grizzard, M.B., Helms, C.M., Barile, M.F., Senterfit, L. and Chanock, R.M. Experimental production of respiratory tract infection with Mycoplasma pneumoniae in rhesus monkeys. J. Infect. Dis. 133: 343-346, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00026-09 LID

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Laboratory and Epidemiologic Studies of Viral Hepatitis Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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99/12	51/12	36/12

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of continuing studies of the chemistry, structure, epidemiology, immunology and pathology of the human hepatitis viruses. The goal of such studies is the control of human viral hepatitis by application of the most appropriate methods, including active and passive immunization, chemotherapy and interdictation of spread of the viruses. Progress: hepatitis A viral antigen was purified from feces and used in newly developed sensitive assays for antibody to the antigen to study the seroepidemiology of hepatitis A virus infection in defined populations. The biophysical and biochemical characterization of hepatitis A viral antigen has begun, and studies of the immunopathology of hepatitis type A in non-human primates, using defined pools of virus, are in progress. An inactivated subunit vaccine for hepatitis type B has been developed and is undergoing extensive tests of safety and efficacy in chimpanzees. A third hepatitis B antigen, e antigen, is being characterized and its relationship to infectivity is being explored. Evidence that populations of hepatitis B viruses may contain defective interfering particles has been obtained, and its finding is being utilized in renewed attempts to isolate the virus. A newly recognized clinical syndrome, type non-A, non-B hepatitis, has been further defined and attempts to identify an etiologic agent intensified.

Project description

Objectives: (1) To collect clinical material of high potential infectivity for use in laboratory studies: (2) To develop and test in vitro procedures and animal systems for use in laboratory studies of viral hepatitis; (3) To propagate the viruses of hepatitis in culture or in experimental animals; (4) To study the biochemistry, biophysics, immunology and epidemiology of hepatitis-associated antigens; (5) To develop vaccines or other control measures for viral hepatitis; (6) To develop chemotherapeutic approaches to the control of chronic viral hepatitis.

Methods Employed

(1) Longitudinal collection of clinical specimens from hepatitis patients; (2) ultracentrifugal, electrophoretic and column chromatographic purification of hepatitis viruses and related antigens; (3) inoculation of non-human primates for the determination of infectivity and clinical spectrum of hepatitis viruses; (4) microscopic examination of hepatitis viruses and virus-infected tissue by fluorescence microscopy and electron microscopy; (5) a variety of tissue culture techniques; (6) a wide variety of immunologic techniques for measuring humoral and cellular immune response.

Major Findings

(1) Hepatitis A:

A. Serology and Epidemiology - A new solid phase radioimmunoassay for the detection of hepatitis A viral antigen and antibody was developed. Using this and other techniques hepatitis A viral antigen was purified from feces of patients acutely ill with type A hepatitis. This antigen was employed in an immune adherence hemagglutination test, modified in this laboratory for use with small quantities of feces-derived hepatitis A antigen, for seroepidemiologic studies of hepatitis A virus infection. In collaborative studies with Bureau of Biologics personnel, San Diego Naval Recruit Center personnel, Dr. James Mosley, Dr. Wolfe Szmuness, Dr. Ian Gust, Dr. Robert McCollum and Dr. Fred Davenport, new knowledge about the ecology of hepatitis A virus was obtained. This included the relationship of age, sex, socioeconomic status, and ethnic and racial origin to the prevalence of hepatitis A virus infection, a preliminary evaluation of the importance of hepatitis A virus in the etiology of "sporadic" (non-epidemic) viral hepatitis in the United States and Australia, laboratory confirmation that hepatitis A virus is important in the etiology of shellfish-associated hepatitis and hepatitis associated with the handling of newly-caught chimpanzees, and the etiologic association of hepatitis A virus with the largest reported epidemic of food-borne hepatitis and two additional smaller outbreaks of viral hepatitis, one occurring in a "commune" and the other in a foundling home. These studies provide the first in-depth analysis of laboratory-confirmed type A hepatitis.

B. Biophysical Characterization - Additional characterization of hepatitis A viral antigen from various sources, including feces, bile and liver, have

been carried out. Virus derived from humans, chimpanzees and marmosets have been analyzed in these studies. Two populations of hepatitis A virus-like particles were found to exist in most fecal specimens; these had buoyant densities in cesium chloride of, respectively, 1.34 and 1.40. In contrast, hepatitis A antigen derived from liver or bile had a density of 1.34. Anti-serum prepared against the "heavy" and "light" populations of hepatitis A antigen particles from feces confirmed that these particles were indeed antigenically related if not identical.

The classification of hepatitis A virus remains in doubt. To resolve this, hepatitis A virus has been purified and is currently being subjected to peptide and nucleic acid analysis. The results of these studies should reveal whether hepatitis A virus is a picornavirus, a parvovirus or a member of a new class of viruses.

C. Transmission studies in non-human primates - A detailed study of hepatitis A virus infection in chimpanzees has been carried out. In addition to an analysis of viralogic and immunologic indicators of infection, the pathology of the disease as determined by light microscopy and electron microscopy has been analyzed in collaborative studies with Dr. Hans Popper and Dr. Fenton Schaffner. Hepatitis type A in the chimpanzee could be distinguished from hepatitis type B by light microscopy, the first time such a pathologic distinction has been reported. Attempts are currently in progress to determine if the distinguishing pathologic lesion characterizes human viral hepatitis types A and B. Viral hepatitis types A and B could also be differentiated under code by electron microscopy.

Because of the shortage of chimpanzees and marmosets for hepatitis type A research, an extensive study of over a dozen species of non-human primates for susceptibility to hepatitis A virus infection has been carried out. Although serologic evidence of infection was found in one or more animals of most of the species tested, none of the animals developed clinical or biochemical evidence of hepatitis and no species was found to be consistently susceptible. It was therefore concluded that chimpanzees and marmosets remain the only non-human primate species suitable for such studies.

2. Hepatitis B.

A. Serology and Epidemiology - Recently an additional hepatitis B antigen, e antigen, and antibody to it were discovered in Sweden. This antigen appears to be virus-specified and has been thought by some to be associated specifically with the Dane particle and long filaments. However, in recent studies with Dr. Gerin, it has been unequivocally shown that e antigen is not on the surface of Dane particles or long filaments and that it is serologically unrelated to hepatitis B surface or core antigens. The main significance of e antigen at present is that its presence in serum appears to be associated with the presence of hepatitis B viral DNA polymerase and a relatively high degree of infectivity. In a recent collaborative study with personnel of the Clinical Center Blood Bank and Dr. Leonard Seeff of the VA, such an association of e antigen with infectivity was confirmed with coded specimens obtained from the recently completed VA hepatitis B hyperimmune globulin (HBIG) study: patients who were

inadvertently stuck with a needle contaminated with HB_s Ag positive blood that was also e antigen positive developed type B hepatitis, whereas those stuck with a needle contaminated with HB_s Ag positive but e antigen negative blood did not develop hepatitis. Surprisingly, this association was almost perfect, irregardless of whether HBIG or standard immune serum globulin was administered. The findings of this study raise serious questions as to the interpretation of the recently completed HBIG studies and suggest that more extensive serologic evaluation of specimens collected from these studies must be carried out before definitive answers as to the efficacy of hepatitis B immune serum globulin can be obtained.

B. Biophysical Characterization - Recently developed in vitro tests for hepatitis B viral DNA polymerase and hepatitis B core antigen have been applied to a more intensive examination of subpopulations of Dane particles in a collaborative study with Dr. Gerin and Dr. Kaplan. The finding of two populations of Dane particle cores, a "heavy" population (buoyant density = 1.36 in CsCl) and a "light" population (buoyant density = 1.31) has been confirmed by demonstrating two equivalent populations of Dane particles (densities of 1.20 and 1.22) from which the respective heavy and light cores could be extracted. Heavy Dane particles often represent a small proportion of the total Dane particle population. On the basis of these findings and analysis of the clinical characteristics of hepatitis B virus infection (long incubation period, cyclic nature of early antigen development and tendency toward persistence and/or chronicity) we have postulated that populations of hepatitis B viruses may consist of fully infectious virus and defective interfering particles. The presence of defective interfering particles might partially explain the inability to isolate hepatitis B virus in vitro. To explore this further, heavy Dane particles are being carefully purified from the more numerous light particles. When completed, the pools of these particles will be titered for infectivity in chimpanzees and will serve as the inoculum for extensive tissue culture isolation studies.

An intensive effort is also being made to purify e antigen for the purpose of preparing potent and highly specific anti-e serum. Such antisera will than be used in the development of sensitive radioimmunoassays for e antigen and antibody and will be fluorscein-labeled in an attempt to determine where in the infected liver cell e antigen is synthesized. We believe that e antigen may represent a non-virion antigen analogous to the "T" antigens or "neo antigens" of other viruses.

C. Hepatitis B Vaccine Development - In collaborative studies with Dr. Gerin and the Infectious Diseases Branch, approximately 10,000 doses each of monospecific subtype adw and subtype ayw hepatitis B subunit vaccine has been prepared from HB_s Ag positive plasma of chronic carriers of the antigen. Immunogenicity tests in guinea pigs have been completed, and both vaccines were found to be highly immunogenic. Safety and efficacy tests of the adw vaccine have begun in chimpanzees and immunogenicity of the vaccine preparation has been confirmed in this species. Tests in chimpanzees of the ayw vaccine will begin shortly. To answer the many questions raised about the safety of such vaccines, extensive tests of humoral and cellular immune response to the

vaccine and to subsequent challenge of vaccinated chimpanzees with live virus are being carried out. A micro test for cell-mediated immunity (CMI) to hepatitis B surface antigen is being used to monitor the immune response in vaccinated chimpanzees. Preliminary evidence indicates that chimpanzees respond to the vaccine by the development of both humoral and cellular immunity. In addition, methods of preserving lymphocytes, developed for use in transplantation immunology, have been adapted to virology: lymphocytes from chimpanzees have been obtained at intervals before and following vaccination and have been frozen for later analysis. We have shown that such lymphocytes retain immunological function after recovery from the frozen state. Thus, it is now possible to do retrospective testing for CMI in a manner analogous to retrospective testing for humoral immunity in virologic studies. Tests for CMI to hepatitis B core antigen and to liver specific proteins are currently being developed and will be used in tests with the preserved lymphocytes when completed.

In cooperation with the Infectious Diseases Branch, suitable human populations for initial safety testing of hepatitis B vaccines have been identified, and baseline seroepidemiologic studies are beginning in these populations. It is hoped that within approximately one year the first human trials of these hepatitis B vaccines will begin.

As part of a continuing analysis of our initial hepatitis B vaccine tests in chimpanzees, a previously vaccinated chimpanzee that was found to be refractory to infection with hepatitis B virus when challenged six months after vaccination was rechallenged eighteen months after vaccination: the animal was still fully resistent to infection. This and other vaccinated chimpanzees will be rechallenged thirty months after the initial vaccination, and the results of this experiment will be available before the first human trials of hepatitis B vaccine.

D. Tissue culture - Based upon our new knowledge about subpopulations of Dane particles, we have begun a more extensive attempt to isolate the hepatitis B virus in vitro. In addition to preparing pools of heavy Dane particles for such experiments, we are searching for tissue culture cell lines and types that can be shown to have receptor sites for hepatitis B virus on their surfaces. This search is being carried out by two means: a modified radioimmunoassay in which the cell itself serves as a "solid-phase" to which Dane particles and surface antigen become adherent and, themselves, serve as a substrate for the attachment of radiolabeled anti-HB_s; and cytoagglutination of cells in the presence of Dane particles and/or hepatitis B surface antigen. In the latter test, neutral red is used to make the cells readily visible.

(3) Non-A, Non-B Hepatitis

A. Serology and Epidemiology - Diagnosis of non-A, non-B hepatitis is one of exclusion, but the availability of sensitive serologic tests for hepatitis A virus and hepatitis B virus infection has permitted the identification of such non-A, non-B cases. In collaborative studies with Dr. Mosley, Dr. Gust and Clinical Center Blood Bank personnel, non-A, non-B hepatitis was found to account for up to 25% of "sporadic" (non-epidemic) clinical hepatitis seen in

Los Angeles and Melbourne, Australia and for up to 90% of clinical and subclinical posttransfusion hepatitis occurring at the Clinical Center Blood Bank.

Attempts to identify an antigen analogous to hepatitis A antigen or hepatitis B antigen but specific for non-A, non-B hepatitis have been unsuccessful to date, as have attempts to transmit this agent to chimpanzees and approximately a dozen species of other non-human primates.

Publications

Purcell, R.H., Feinstone, S.M., and Kapikian, A.Z.: Recent advances in hepatitis A research. In Greenwalt, T.J., Jameison, G.A. (Ed.): Proceedings of a Symposium on Transmissible Disease and Blood Transfusion. New York, Grune & Stratton, 1975, pp. 11-26.

Purcell, R.H.: Current status of diagnostic tests for viral hepatitis. In Mathieu and Kahan (Eds.): Immunological Aspects of Anesthetic and Surgical Practice. New York, Grune & Stratton, 1975, pp. 205-255.

Purcell, R.H.: Current understanding of hepatitis B virus infection, and its implications for immunoprophylaxis. In Perspectives in Virology IX. Chapter 3, pp. 49-76 1975.

Kapikian, A.Z., Feinstone, S.M., Purcell, R.H., Wyatt, R.G., Thornhill, T.S., Kalica, A.R., and Chanock, R.M.: Detection and identification by immune electron microscopy of fastidious agents associated with respiratory illness, acute non-bacterial gastroenteritis and hepatitis A. In Perspectives in Virology IX. Chapter 2, pp. 9-47, 1975.

Purcell, R.H.: The nature of the hepatitis B virus. In Logan, G.E. (Ed.): Proceedings of the First Australasian Hepatitis Symposium. Melbourne, Australia, Abbott Laboratories, 1975, pp. 93-100.

Purcell, R.H.: Overview and summing up. In Logan, G.E. (Ed.): Proceedings of the First Australasian Hepatitis Symposium. Melbourne, Australia, Abbott Laboratories, 1975, pp. 293-296.

Moritsugu, Y., Gold, J.W.M., Wagner, J., Dodd, R.Y., Purcell, R.H.: Hepatitis B core antigen: Detection of antibody by radioimmunoprecipitation. J. Immunol. 114: 1792-1798, 1975.

Purcell, R.H., Dienstag, J.L., Feinstone, S.M., and Kapikian, A.Z.: Relationship of hepatitis A antigen to viral hepatitis. Amer. J. Med. Sci. 270: 61-71, 1975.

Dienstag, J.L., Routenberg, J.A., Purcell, R.H., Hooper, R.R., Harrison, W.O.: Foodhandler-associated outbreak of viral hepatitis type A: An immune electron microscopic study. Am. Intern. Med. 83: 647-650, 1975.

Purcell, R.H., Gerin, J.L.: Hepatitis B subunit vaccine: A preliminary report of safety and efficacy tests in chimpanzees. Amer. J. Med. Sci. 270: 395-399, 1975.

Reeves, W.C., Peters, C.J., and Purcell, R.H.: The epidemiology of hepatitis B antigen among Panamanian Cuna indians. J. Infect. Dis. 24: 873-875, 1975.

Peterson, J.M., Dienstag, J.L., and Purcell, R.H.: Immune response to hepatitis viruses. In Notkins, A.L. (Ed.): Viral Immunology and Immunopathology, Academic Press, 1975, pp. 213-236.

Dienstag, J.L., Feinstone, S.M., Purcell, R.H., Hoofnagle, J.H., Barker, L.F., London, W.T., Popper, H., Peterson, J.M., and Kapikian, A.Z.: Experimental infection of chimpanzees with hepatitis A virus. J. Infect. Dis. 132: 532-545, 1975.

Purcell, R.H., Wong, D.C., Moritsugu, Y., Dienstag, J.L., Routenberg, J.A., and Boggs, J.D.: A microtiter solid-phase radioimmunoassay for hepatitis A antigen and antibody. J. Immunol. 116: 349-356, 1976.

Barker, L.F., Maynard, J.E., Purcell, R.H., Hoofnagle, J.H., Berquist, K.R., London, W.T., Gerety, R.J., and Krushak, D.H.: Hepatitis B virus infection in chimpanzees: titration of subtypes. J. Infect. Dis. 132: 451-458, 1975.

Maynard, J.E., Lorenz, A.D., Bradley, D.W., Feinstone, S.M., Krushak, D.H., Barker, L.F., and Purcell, R.H.: Review of infectivity studies in non human primates with virus-like particles associated with MS-1 hepatitis A. Am. J. Med. Sci. 270: 81-85, 1975.

Barker, L.F., Maynard, J.E., Purcell, R.H., Hoofnagle, J.H., Berquist, K.R., and London, W.T.: Viral hepatitis, type B in experimental animals. Am. J. Med. Sci. 270: 189-195, 1975.

Knodell, R.G., Conrad, M.E., Dienstag, J.L., Bell, C.J.: Etiologic spectrum of post-transfusion hepatitis. Gastroenterology 69: 1278-1285, 1975.

Murphy, B.L., Peterson, J.M., Smith, J.L., Gitnick, B.L., Auslander, M.O., Berquist, K.R., Maynard, J.E., and Purcell, R.H.: Correlation between fluorescent antibody detection of hepatitis B core antigen (HB_c Ag) in liver biopsies and the presence of e-antigen in serum. Infect. Immun. 13: 296-297, 1976.

Gerin, J.L., Ford, E.C., and Purcell, R.H.: Biochemical characterization of Australia antigen: evidence for defective interfering particles of hepatitis B virus. Am. J. Pathol. 81: 651-668, 1975.

Dienstag, J.L., and Purcell, R.H.: An antigen associated with viral hepatitis, type A: clinical implications. Comprehensive Therapy, in press, 1976.

Moritsugu, Y., Dienstag, J.L., Valdesuso, J., Wong, D.C., Wagner, J.A., Routenberg, J.A., and Purcell, R.H.: Purification of hepatitis A antigen from feces and detection of antigen and antibody by immune adherence hemagglutination. Infect. Immun. 13: 898-908, 1976.

Alter, H.J., Holland, P.V., and Purcell, R.H.: The emerging pattern of post-transfusion hepatitis. Am. J. Med. Sci. 270: 329-334, 1975.

Madden, D.L., Dietzman, D.E., Matthew, E.B., Sever, J.L., Lander, J.J., Purcell, R.H., Rostafinski, M., and Mata, A.: Epidemiology of hepatitis B virus in an institution for mentally retarded persons. Am. J. Ment. Deficiency 80: 369-375, 1976.

Kapikian, A.Z., Dienstag, J.L., Purcell, R.H.: Immune electron microscopy as a method for the detection, identification, and characterization of agents not cultivable in an in vitro system. In Russel, P. (Ed.): Manual of Clinical Immunology, American Society for Microbiology, Washington, D.C., in press, 1976.

Dienstag, J.L., Alling, D.W., Purcell, R.H.: Quantitation of antibody to hepatitis A antigen by immune electron microscopy. Infect. Immun., in press, 1976.

Purcell, R.H., Alter, H.J., Dienstag, J.L.: Non-A, non-B hepatitis. Yale J. Biol. Med., in press, 1976.

Schulman, A.N., Dienstag, J.L., Jackson, D.J., Hoofnagle, J.H., Gerety, R.J., Purcell, R.H., Barker, L.F.: Hepatitis A antigen particles in chimpanzee liver, bile, and stool. J. Infect. Dis., in press, 1976.

Murphy, B.L., Peterson, J.M., Ebert, J.W., Berquist, K.R., Maynard, J.E., and Purcell, R.H.: Immunofluorescent localization of hepatitis B antigens in chimpanzee tissues. Intervirology, in press, 1976.

Berquist, K.R., Peterson, J.M., Murphy, B.L., Ebert, J.W., Maynard, J.E., and Purcell, R.H.: Hepatitis B antigens in serum and liver of chimpanzees infected with hepatitis B virus. Infect. Immun. 12: 602-605, 1975.

Madden, Matthew, Dietzman, Purcell, and Sever: Hepatitis and Down's Syndrome. Am. J. Ment. Deficiency 80: 401-406, 1976.

Dienstag, J.L., Gust, I.D., Lucas, C.R., Wong, D.C., Purcell, R.H.: Mussel-associated viral hepatitis type A: serological confirmation. Lancet 1: 561-564, 1976.

Kaplan, P.M., Ford, E.C., Purcell, R.H., and Gerin, J.L.: Demonstration of subpopulations of Dane particles. J. Virol. 17: 885-893, 1976.

Alter, H.J., Purcell, R.H., Holland, P.V., Feinstone, S.M., Morrow, A.G., and Moritsugu, Y.: Clinical and serological analysis of transfusion-associated hepatitis. Lancet 2: 838-841, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00027-09 LID

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Basic Studies on Mycoplasmas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.G. Tully Head, Section of Mycoplasmas LID NIAID

COOPERATING UNITS (if any)
Bureau of Biologics, FDA, Bethesda, Md. (Dr. M.F. Barile)
U.S. Dept. Agriculture, Beltsville, Md. (Dr. R.F. Whitcomb)
Wistar Institute, Philadelphia, Pa. (Dr. H.F. Clark)
Dr. R.M. Cole (Ser. No. Z01 AI 00004-16 LSD) W.L. Cline (Bio. Lab. Tech. LID)

LAB/BRANCH
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives cover both basic and applied aspects of mycoplasmas and related wall-free prokaryotes, including their neurotoxins and other biological factors involved in virulence, their immunological interrationships, and their possible role in human infection or in diseases of uncertain etiology. Current projects of interest involve the characterization of a newly-isolated spiroplasma, its pathogenicity for primates and other animal hosts, and sero-epidemiological studies on possible host distribution of the organism, including serological tests on paired human sera from a variety of neurological diseases and normal human sera from selected age groups.

Project Description

Studies on the suckling mouse cataract agent (SMCA) reported last year have been continued. This agent, previously thought to be a slow virus, was found to have some serological cross reactions with a group of plant and insect mycoplasmas (spiroplasmas). SMCA, and a second tick-derived agent (GT-48), were grown in the developing chick embryo and both allantoic and amniotic fluids contained numerous motile, helical forms typical of spiroplasmas. Subsequent morphological studies, employing both darkfield and electron microscopy, identified the agent as completely similar to other helical, wall-free prokaryotes (spiroplasmas). The newly-recovered organism turned out to be rather unique spiroplasmas since they possessed capabilities of growing at temperatures from 30° to 37°C and were lethal for vertebrates. Previously described spiroplasmas have a fairly sharp temperature optimum at 29-30°C and have exhibited no pathogenicity for hosts outside of plants and insects. Additional serological tests with egg-derived SMCA antigens and antisera against other known spiroplasmas confirmed our earlier findings of a partial serological relationship among these organisms. The present findings indicate that these organisms represent a completely new class of microbial agent, not capable of being cultured on the usual laboratory media and not detected by conventional serological tests with currently-known agents. Their ability to produce disease in vertebrates with prolonged persistence of the organisms in host tissues suggests a search for their presence in human diseases of unknown etiology. (Clark, Wistar Inst.; Whitcomb, USDA)

SMCA and GT-48 organisms recovered from allantoic fluids contained numerous virions characteristic of those designated SV-C3 (spiroplasma virus-citri 3), and previously observed in Spiroplasma citri. Viral infections of SMCA and GT-48 have also been noted in organisms adapted to cell-free culture. Although only the C3 virus (a short-tailed bacteriophage-like organism) has been noted in these spiroplasmas, additional studies are in progress on the occurrence of other viral forms and what role viruses may play in the pathogenicity of SMCA. A full understanding of the viral infection will require some technical developments, especially the availability of susceptible (or perhaps virus-free) indicator strains for plaque titrations. The presence of viruses in SMCA offers further evidence for the direct association of viruses with all known spiroplasmas and provides additional tools to study the relationship of these viruses to other mycoplasma viruses (Cole, LSD)

SMCA ant GT-48 isolates were successfully cultivated on 2 of 20 different media formulations prepared from conventional mycoplasma broth supplemented with various tissue culture media components. Growth did not occur on conventional mycoplasma medium nor on other media used for spiroplasma cultivation. Titers of 10^{8} to 10^{9} color-changing units/ml (or comparable colony-forming units/ml in subsequent cultures adapted to agar) were obtained after several passages. Cultures carried through terminal dilution techniques to greater than 10^{-25} of the original inoculum indicated adaptation of the agents to the specific cell-free medium. Virulence tests with cultured SMCA chick embryos revealed egg LD_{50} titers of $10^{9.0}$/0.5 ml or greater. Cultured

SMCA also produced the expected host symptoms of cataracts and central nervous system (CNS) disease in suckling rats. Thus, early passage of the agents in culture media did not alter the pathogenicity to susceptible hosts. The organisms were maintained at both 30°C and 37°C in continuous culture. Our success in recovery of these spiroplasmas only on specially-formulated media indicates why these organisms were missed by conventional cultural procedures--observations which suggest as yet unrecognized mycoplasmas might well be present in, and associated with, pathological conditions of humans and other hosts (Whitcomb, USDA; Clark, Wistar Inst.)

Biological and serological characterization of SMCA and GT-48 spiroplasmas were initiated in attempts to establish the full relationship of these organisms to other spiroplasmas and to provide a basis for subsequent pathological and serological studies. Both isolates were triple cloned following ultrafiltration and growth on solid media. Studies relating the occurrence of disease symptoms in susceptible hosts following challenge with cultured SMCA and subsequent recovery in culture of the same organism have been performed successfully. Thus, Koch's postulates on SMCA spiroplasmas have been fulfilled. Additional studies on primary recovery of SMCA from infected allantoic fluids in various media formulations indicated that a medium sufficient to maintain the agent in continuous culture was not always adequate to recover the agent from animal tissues. Further biological and serological comparisons of SMCA spiroplasmas and other spiroplasmas are in progress. (Whitcomb, USDA)

A complement-fixation test with cultured SMCA antigen has been employed in seroepidemiological studies. In general, about 50% of normal human sera from both children and young adults possess low levels (1:4 to 1:8) of antibody to SMCA. A selection of paired sera from 47 children with non-specific CNS disease (mostly aseptic meningitis) revealed 10/47 with 4-fold or greater increases in antibody titer. One patient with Stevens-Johnson syndrome had a 16-fold increase in titer but several other children with this diagnosis did not exhibit SMCA antibody titers. A small group (13) of age-matched, paired control sera indicated no evidence of comparable 4-fold titer increases. CF tests carried out on a group of sera from children with confirmed Reye's syndrome did not point to any direct association between this disease and SMCA. A group of 13 paired sera from young adults (military age) with CNS disease were tested in the CF procedure and 3/13 had 2-fold rises in antibody in convalescence and 1 individual exhibited a 4-fold rise. Additional serological tests on select sera from various diseases are planned, along with attempts to enhance the sensitivity of the test procedures through application of radioimmunoassay techniques (Cline, LID).

Preliminary studies on the primate pathogenicity of early subcultures of SMCA have been performed. Primary SMCA challenge of a Cynamologous monkey with SMCA via intracerebral, intrathecal, and intravenous routes revealed obvious CNS symptoms, including disorientation, incoordination, and some transient posterior paralysis. While these symptoms regressed over a 2 week period, there was some residual low-level incoordination apparent 1 month after challenge. A control monkey receiving broth by the stated routes did not show

any changes. Serological analysis of the response in the challenged animal showed a 32-fold increase in CF titer at 4 weeks following challenge. These tests also revealed a significant pre-existing CF titer to SMCA (1:128) in the challenged monkey, a factor which might well have conditioned the response noted. Further testing of normal Cynamologous monkeys indicated about 1/3 of 20 animals had rather high (1:32 to 1:64) CF titers to SMCA, while twenty sera from normal Rhesus monkeys did not show SMCA antibody. Subsequent challenge studies are in progress, employing Cynamologous monkeys previously screened in the CF test for absence of SMCA antibody. (Barile, FDA; Cline, LID)

Publications

Tully, J.G., Whitcomb, R.F., Williamson, D.L., and Clark, H.F.: Nature 259: 117-120, 1976.

Tully, J.G., and Razin, S.: The Mollicutes (mycoplasmas). In Laskin, A.I. and Lechevalier, H.A. (Eds.): Handbook of Microbiology, 2nd edition Cleveland, Chemical Rubber Co., 1976, volume 1 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00028-18 LID

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Study of Respiratory Viruses and Mycoplasmas in Volunteers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Dr. Charles M. Helms	NIAID/LID	Medical Officer
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
36/12	12/12	24/12

SUMMARY OF WORK (200 words or less - underline keywords)

The response of volunteers to ts mutants of influenza A virus and M. pneumoniae was evaluated. The influenza A/Georgia/1974 ts-1[E] recombinant was shown to be safe, satisfactorily attenuated and antigenic in adults who lacked serum HI antibody but possessed neuraminidase immunity. This recombinant represents the 3rd influenza A virus into which the ts-1[E] lesions have been transferred. Significantly, each of the 3 ts-1[E] recombinants was suitably attenuated and antigenic. This experience supports the thesis that influenza A viruses can be predictably attenuated by transfer of a defined set of ts genetic lesions.

Ts H43 mutant of M. pneumoniae was found to retain some residual virulence. An occasional seronegative volunteer developed mild afebrile bronchitis. The ts-H28 mutant of M. pneumoniae, which is more temperature sensitive than t-H43, had low infectivity for seronegative adults.

Project Description

Objectives: (1) To evaluate temperature-sensitive mutants and recombinants of influenza A virus for evidence of attenuation and antigenicity in volunteers; (2) to characterize the serum and local respiratory tract anti-neuraminidase and anti-hemagglutinin antibody responses of man to infection with wild type or attenuated influenza A virus; (3) to evaluate the infectivity and attenuation of ts mutants of M. pneumoniae.

Major Findings

(A) Evaluation of ts-1[E] recombinants influenza A viruses

Studies are in progress with an influenza A/Georgia/101/1974-ts-1[E] recombinant which contains the 2 ts-1[E] lesions and has a 38°C shutoff temperature for plaque formation. A total of 32 volunteers lacking HAI antibody were administered $10^{6.5}$ $TCID_{50}$/ml. Only 3 volunteers developed mild upper respiratory symptoms without fever and thus the vaccine was considered suitably attenuated. Twenty-three volunteers shed virus but the amount was reduced 100 to 1000 fold compared to wild type Georgia/74 virus. Each of the viruses recovered from the infected volunteers retained the ts phenotype. Twenty-eight of the 32 volunteers developed a rise in serum HAI antibody. The neuraminidase-inhibition and nasal wash neutralization responses are currently being evaluated. Nevertheless, these data, provide strong support for our central hypothesis, namely any virus within the H3N2 subtype that possesses the 2 ts-1[E] lesions and a 38° shutoff temperature will be suitably attenuated for adults who possess some neuraminidase immunity.

(B) Evaluation of a wild type virus with a passage history similar to the ts recombinant viruses

Each of the ts recombinant viruses administered to volunteers was virulent than its wild type parent. The ts viruses, however, underwent a greater number of passages in tissue culture than their low passage, uncloned wild type parent. Since influenza A viruses can be attenuated by repeated passage in tissue culture from a heterologous host, it is possible that this effect may have played some role in the attenuation of the ts recombinants. To examine this question, a wild type Udorn/72 virus suspension was cloned and passaged in a manner comparable to its ts recombinants and then evaluated in adult seronegative volunteers. The clinical, virologic, and immunologic response to this wild type (BK 6; egg-1) virus was similar to that of the uncloned wild type virus (BK-3), but was significantly different from that of the Udorn/72 single lesion clone 13 ts (39°C) and double lesion clone 24 (38°C shutoff temperature) recombinants which were studied in their BK-5 egg 1 passage. Even at a concentration of $10^{7.0}$ to $10^{7.7}$ $TCID_{50}$ per volunteer the ts viruses produced illness less frequently and the illness which occurred was less severe. In addition, virus was shed less often and in lower titer. This suggests that attenuation of temperature sensitive viruses is not a result of passage in heterologous tissue culture but is a consequence of the acquisition of one or more ts lesions.

(C) Evaluation of H0N1 wild type viruses in man for virulence

Previous evaluation of Hong Kong/68 and Udorn/72-ts-1[E] recombinants in doubly seronegative children revealed that these viruses retained mild reactogenicity and exhibited a low level of genetic instability. These properties made the ts-1[E] viruses unsuitable as vaccine candidates for this population. The possibility exists that the administration of ts-1[E] recombinants to doubly seronegative adults (i.e., at the time of a pandemic shift involving both surface antigens) might also be associated with mild reactogenicity and genetic instability. In an attempt to study this question, we transferred the ts-1[E] lesions into an H0N1 (1943) wild type virus with the intention of evaluating such recombinants in doubly-seronegative adults (i.e. individuals born since 1947 when H0N1 viruses stopped circulating). However, before H0N1 recombinants could be assessed for attenuation it was necessary to establish that the wild type virus was virulent. Thus, a total of 20 doubly seronegative volunteers were given 10^3, 10^4, 10^5, or 10^6 $TCID_{50}$ of the wild type H0N1 virus (E9, BK, E3, Hul, BK4). Only 2 of 6 volunteers who received the 10^5 dose were infected and in both instances infection was asymptomatic. Persons who received the other inocula were not infected. Comparable doses of an H3N2 wild type virus have infected 90% of volunteers who lacked antibody for the H3 antigen although such persons possessed some neuraminidase immunity. In addition, illness occurred in greater then 50% of such individuals. The response of doubly seronegative adults to the H0N1 wild type virus indicated that it was over-attenuated for man and could not be used to evaluate the effect of the ts-1[E] lesions on virulence. Currently an A/Eng/1/53 (H1N1) wild type virus is being prepared for this purpose.

(D) Evalution of interferon and cell-mediated immune response to wild type influenza A virus

The interferon evaluation was done in collaboration with Dr. Samuel Baron of LBV, NIAID. Three wild type antigenic variants of the H3N2 subtype (representing the years 1968, 1972 and 1974) were examined for their sensitivity to interferon and for their ability to induce local respiratory tract interferon in volunteers. In addition, the time of appearance of symptoms in infected volunteers was compared with the patterns of virus shedding and interferon production. The 3 virus strains could not be differentiated as regards sensitivity to interferon or ability to stimulate nasopharyngeal wash interferon in volunteers. Illness, peak virus shedding, and peak interferon response each occurred within a 26 hour period. These findings imply that interferon or its inducers theoretically could be protective if applied prophylactically but would be less effective when used therapeutically.

The evaluation of the cell-mediated immune response was done in collaboration with Dr. Raphael Dolin, NIAID, LCI. Cell mediated immune responses were examined in 19 normal volunteers following intranasal administration of one of 3 influenza A virus strains. Eight volunteers manifested respiratory tract illness, along with a four-fold rise in serum antibody and/or virus shedding. Peripheral venous blood samples were obtained prior to, 2 days, 5 days, and 4 weeks after challenge. During acute illness infected volunteers manifested a lymphopenia, which persisted up to 4 weeks after challenge.

The lymphopenia involved T, B and null cells. Blastogenic responses of lymphocytes to stimulation with PHA, ConA, and SKSD were depressed during acute illness, and remained below pre-challenge responses for PHA and ConA at 4 weeks after infection. Blastogenic responses to influenza A(H3N2) antigens were detected in lymphocytes from initially sero-negative volunteers 4 weeks after infection with influenza A/Udorn/307/72(H3N2). The blastogenic response correlated with the level of serum hemagglutination-inhibiting antibody. Thus, influenza A virus infection in man resulted in the stimulation of a specific cell mediated immune response while at the same time non-viral, non-specific cell mediated responses were suppressed. The specific anti-influenza A cell mediated immune response to an A/Victoria/3/75 wild type virus will be further evaluated using cytotoxicity and blastogenesis as indicators of immunity. Cells infected with the A/VIC/3/75, H Equi 1 $N2_{75}$, $H3_{75}$ N equi 1, and H equi 1 N equi 1 viruses and inactivated antigens for the study of bastogenic response are being prepared to evaluate which antigen of the influenza A virus the cell-mediated immune response is directed against.

(E) Evaluation of ts Mutants of Mycoplasma pneumoniae

In the past year, the ts H43 mutant has undergone further evaluation in adult volunteers and studies using the most temperature-sensitive of the mutants, ts H28, were initiated.

To date, a total of 75 volunteers have received ts H43 in doses ranging from 10^4-10^8 CFU. Seven of the 8 volunteers who received 10^8 CFU intranasally were successfully infected as evidenced by a serologic response and/or shedding of the ts-H43 mutant. Two of these volunteers, however, developed mild afebrile bronchitis for which antibiotic therapy was not deemed necessary. In an attempt to decrease the residual virulence of this mutant, a reduced dose was administered to a total of 60 healthy volunteers. Forty-two seronegative (serum metabolism-inhibiting antibody titer $\leq$1:2) and 4 seropositive individuals (titer 1:2) received $10^{6.0-7.0}$ CFU of ts H43 by intranasal administration of coarse-particle aerosol. Ninety-five percent of the seronegative and 50% of the seropositive volunteers showed evidence of infection. Isolates obtained from the volunteers uniformly retained the ts phenotype of the candidate vaccine strain indicating that the mutant was genetically stable in man. While 37 persons underwent an asymptomatic infection with this inoculum of ts H43, 9 developed one or more signs of minor respiratory tract infection. In 6 cases, illness was confined to the upper respiratory tract; 3 volunteers, however, developed afebrile bronchitis.

An additional 14 volunteers were given $10^{4.0-4.9}$ CFU of ts H43. Four of 7 volunteers who received $10^{4.9}$ CFU intranasally were infected as compared to none of 7 who received $10^{4.0}$ CFU. One volunteer developed afebrile bronchitis one day after inoculation while the other volunteers remained well. In view of the early and unusual onset of symptoms and the observation that the former volunteer did not demonstrate evidence of infection (serologic response and/or shedding of the organism) the etiology of his 4-day illness remains uncertain. Additional studies are planned to clarify the effect of this dose range of ts H43 (10^4-10^5 CFU/volunteer).

The effect of oral administration of ts H43 was evaluated in 7 volunteers using a large inoculum (10^8 CFU/volunteer). Only one person developed a detectable serum antibody response; none shed the organism. On the basis of this limited trial, it does not appear that oral feeding of this mutant is an effective mode of administration.

In addition, studies in volunteers have been initiated with the ts H28 mutant of M. pneumoniae. This is the most defective and temperature sensitive of the ts mutants as indicated by studies in vitro. Of the 23 volunteers inoculated intranasally with 10^6 CFU, 4 (18%) developed a serologic response; none shed the organism. One of the infected individuals developed a mild upper respiratory tract illness.

Studies anticipated next year include the further evaluation of ts H43 (10^4-10^6 CFU/volunteer) administered by nasal drops. It is also our intention to study M. pneumoniae mutant ts 640 which has undergone 15 passages in artifical broth medium. Initial studies in volunteers using 10^7 CFU of the 14th passage of this mutant suggested that it was highly infective (100%) but retained an unacceptable measure of residual virulence (2 of 11 volunteers developed febrile bronchitis). The effect of reduced dose (<10^6 CFU/volunteer) and oral administration will be studied in healthy adult volunteers to determine if a silent infection can be established.

Publications

Murphy, B.R., Richman, D.D., Chalhub, E.G., Uhlendorf, C.P., Baron, S., and Chanock, R.M.: Failure of attenuated temperature-sensitive influenza A (H3N2) virus to induce heterologous interference in humans to parainfluenza type 1 virus. Infect. Immun. 12: 62-68, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00029-02 LID

PERIOD COVERED

July 1, 1975 though June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on ts Mutants of Streptococcus pneumoniae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Dr. Michael B. Grizzard, LID, NIAID, Research Associate
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Dr. Allen Cheever, LPD, NIAID) Medical Officer
Dr. Gerald Schiffman, Downstate Med. Ctr. S.U.N.Y.,

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SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
30/12	12/12	18/12

SUMMARY OF WORK (200 words or less - underline keywords)

Although parenterally administered capsular polysaccharide vaccine is highly effective in preventing pneumococcal pneumonia in adults, it appears less effective in preventing otitis media in infants. Thus there is a need to explore alternate approaches to prevention of this type of ear disease. We have selected ts mutants of S. pneumoniae which grow vigorously at 32°C, but which do not replicate efficiently at 38°C. Mutants of this type should replicate in the nasopharynx and stimulate local antibody, but should not produce disease in the middle ear or lower respiratory tract because of their failure to grow to high titer at the restrictive temperature of these areas. Ts mutants of type 1 S. pneumoniae were selected following exposure of virulent (ts^+) organisms to nitrosoguanidine. Each mutant resembled the ts^+ parent in bacteriologic and serologic properties, but was restricted in capacity to form colonies at 38°C. When inoculated I.P. into mice, 11 of 13 mutants were attenuated and induced homologous resistance. Three mutants were also attenuated and immunogenic following administration I.P. to hamsters. Attenuation in hamsters may be related to decreased growth and survival of ts organisms at body temperature. Two mutants were completely attenuated for hamsters when administered intranasally and induced resistance to subsequent challenge with ts+ organisms by the same route.

Project Description

Objectives: S. pneumoniae, the primary bacterial pathogen of otitis media, continues to be a major cause of hearing loss in early childhood. Despite the established effectiveness of the polyvalent pneumococcal polysaccharide vaccine in preventing pneumococcal pneumonia in adults, preliminary studies with a series of pneumococcal polysaccharides have indicated that they are less antigenic in infants and young children than in adults. Recent studies have demonstrated that local secretary IgA antibody is secreted by goblet cells lining the middle ear and upper Eustachian tube and it is now evident that certain bacterial species are capable of inducing a local antibody response. We have sought to take advantage of the temperature differential in the respiratory tract and have selected mutants of S. pneumoniae which grow vigorously at 32°C, the temperature of the upper respiratory tract, but which do not replicate efficiently at the temperature of the lower respiratory tract, 37°C. Mutants of this type would be able to replicate efficiently in the nasopharynx and stimulate the production of local antibody but would not retain the capacity to produce disease in the middle ear or lower respiratory tract because of their failure to grow to high titer at the restrictive temperature of these areas.

Progress

Thirteen temperature-sensitive (ts) mutants of type 1 S. pneumoniae were selected following exposure of the virulent wild-type (ts^+) organisms to the chemical mutagen nitrosoguanidine. Each mutant resembled the ts^+ parent in properties of α-hemolysis, bile solubility, optichin sensitivity, antibiotic sensitivity and serotype. Unlike the ts^+ parent, however, each ts mutant was restricted in capacity to form colonies on blood agar at 38°C. With the exception of one mutant (ts 8), there was a correlation between the degree of temperature-sensitivity of a mutant and its genetic stability. The basis of the exception of mutant ts 8 to the general correlation of temperature-sensitivity and genetic stability is currently under study.

When inoculated intraperitoneally into mice, 11 of 13 mutants were attenuated and induced homologous resistance. Three mutants (ts 1, ts 3 and ts 4) were also studied in hamsters and were found to be attenuated and immunogenic following intraperitoneal injection. Study of the behavior of mutants ts 1, ts 3 and ts 4 in the blood of hamsters suggested that attenuation may be related, in part, to decreased growth and survival of ts organisms at body temperature. Mutants ts 1 and ts 4 were completely attenuated for hamsters when administered intransally and induced significant resistance to subsequent challenge with wild-type organisms by the same route. The basis of this acquired resistance in the hamster is being pursued at present.

Significance of the Program to the Institute

Pneumococcal infection is an important cause of respiratory tract illness, central nervous system disease, and otitis media with subsequent hearing loss in young children. Although polysaccharide vaccine appears to be

highly effective in preventing pneumococcal pneumonia in adults there is evidence that parenteral administration of a polysaccharide vaccine has considerably less effect in preventing otitis media in infants. For this reason there is a need to explore alternate approaches to prevention of this type of ear disease.

Local administration of ts mutants of type 1 S. pneumoniae to hamsters may provide a model for evaluating the potential of live vaccines for the prevention of pneumococcal disease.

Proposed Course of Study

We plan to study the complete set of ts mutants of S. pneumoniae in hamsters and mice utilizing the nasopharyngeal route of immunization and challenge.

Studies to be completed next year include a more extensive evaluation of the relationship between temperature-sensitivity of the mutants and bacteremia following intraperitoneal inoculation in hamsters. In addition, an analysis of the comparative growth of ts mutants in the lungs, blood, and nasal turbinates following intranasal inoculation will be initiated.

Publications

Helms, C.M., Grizzard, M.B., and Chanock, R.M.: Temperature-sensitive mutants of type 1 Streptococcus pneumoniae: Preparation, characterization, and evidence for attenuation and immunogenicity. J. Infect. Dis., in press.

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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID
July 1, 1975 - June 30, 1976

Dr. Wallace P. Rowe
Chief, Laboratory of Viral Diseases

Murine Leukemia Viruses. The major program effort of LVD is focused on these complex and important agents. This program is broadly based, encompassing animal genetics, tissue culture virology, and biochemical (primarily nucleic acid hybridization) techniques, and involving collaborative studies with a number of other laboratories. The emphasis in this program continues to be the natural history of these viruses, including the questions of the origins of the viral genome, which we have shown to be carried as chromosomal genes, the factors regulating expression of these genomes, and the relation of their expression and of the host genotype to tumorigenesis.

Continuing studies of the genetics of endogenous C-type viral genomes in the mouse have shown that two mouse strains with highly inducible loci, the PL and RF strains, carry only one locus for ecotropic virus. This is in contrast to the three high-virus strains previously studied (AKR, C58, and C3H/Fg), which have two to four such genetic loci (Ishimoto, Risser, Rowe). The mouse strain which we have found to be inducible for B-tropic virus, B10.Br/Li, also appears to contain one virus-inducing locus, but to have another type of locus as well, which allows this locus to be expressed as both N- and B-tropic virus; in the absence of this second locus, the virus-inducing locus is expressed only as B-tropic virus. This finding represents a new level of complexity in the genetic control of the expression of these viruses, but it may provide the key to understanding the basis of N- and B-tropism (Rowe, Hartley). Genetic transmission of xenotropic virus inducibility has been followed in several crosses, and in all cases it segregates as a single gene (Hartley, Rowe).

An important finding has been made which points to a relationship between xenotropic virus expression and lymphomagenesis. The Sloan-Kettering group (Old, Stockert, and Boyse) have observed a marked increase in amount of certain C-type viral antigens in the preleukemic AKR thymus occurring at about 6 months of age. In collaborative studies with the Sloan-Kettering group, we have found that this increase of antigen is accompanied by appearance of large amounts of xenotropic virus, but with no change in amount of ecotropic virus (the classical Gross-type virus); AKR lymphomas also show large amounts of xenotropic virus (Hartley).

Of possible relevance to this is our finding that phenotypic mixing occurs between ecotropic and xenotropic murine C-type viruses. In harvests of mixed infection we can detect particles with the ecotropic virus genome and the host range of the xenotropic virus, and other particles with the reciprocal properties. This shows that xenotropic virus can in fact replicate in mouse cells if it can be gotten past some early steps of the infection that are determined by the components of the virion. This may well occur in vivo in mice producing

both types of virus. These studies show that the N- or B-tropism of the ecotropic virus is shared by the phenotypically mixed particles with the xenotropic genomes, indicating that the Fv-1 restriction must involve a stage of infection prior to integration of the provirus (Ishimoto, Rowe, Hartley).

A system has been developed for doing segregation analysis of the genetic basis for sensitivity to lymphomagenesis in adult mice inoculated with Abelson B-cell lymphoma virus. The segregation ratios indicate that two genes are involved, both of which are recessive for resistance (Risser, Rowe; Potter, NCI).

Interferon therapy in monkeys, chimpanzees and man. The new interferon inducer, a stabilized derivative of poly I.poly C containing poly L-lysine and carboxymethyl cellulose was shown last year to be a good interferon inducer and antiviral agent in non human primates. Control of Russian spring summer encephalitis in monkeys and inhibition of hepatitis B in chimpanzees was demonstrated during the current year. In addition, the drug has proven to be a good interferon inducer in man, both topically when applied as drops to the eye, and systemically, during a phase 1 study in cancer patients and patients with serious viral disease. The drug is effective against superficial vaccinia infection in rabbits when applied as an ointment. A double blind study is underway on herpes genitalis in man (Levy and Lvovsky).

The drug also is a very potent immune adjuvant when given in monkeys along with a killed Venezualan equine encephalitis vaccine, increasing both the titer of antibody against the virus and the length of time the antibody can be detected (Levy).

A new class of compounds, the radioprotective aminothiols, has been shown to be capable of inducing interferon when administered orally to monkeys (Lvovsky and Levy).

Mechanism of action of interferon. The primary antiviral activity of interferon is mediated by an alteration of uninfected cells, such that cell messenger RNA is translated, but translation of viral mRNA is restricted. It was shown that this ability to distinguish among different classes of mRNA resides in 3 factors, a) modified ribosomes, b) modification of cell mRNA though the addition of an additional structure to preexisting mRNA, c) a modification of tRNA that involves in some way, an alteration in secondary structure (Levy and Riley).

Large scale production of human interferon. In studies by C. Buckler in collaboration with investigators from LCB, NIHMDD and the Department of Virus Production, FCRC, methods for the large scale production of human interferon have been developed. These studies are directed toward eventually producing 3000 gallon runs, needed for obtaining milligram quantities of human interferon. This will be used as the starting material for purification and for the determination of the chemical and physical properties of one type of human interferon. The chemical synthesis of interferon requires a prior determination of its primary amino acid sequence.

5-iododeoxyuridine (IdU) enhancement of viral replication. Replication of several RNA and DNA viruses is potentiated in cell cultures which have been

pretreated with the pyrimidine analogue IdU. Enhanced viral replication occurs in a number of different cell types including primary cell cultures, strains and lines. In the case of viruses which normally replicate in an efficient manner IdU appears to enhance the rate of replication without increasing either the number of cells infected or the final virus yield per infected cell (Green, Baron and Levy).

Rapid microtiter plaque assay of vesicular stomatitis virus (VSV). VSV rapidly produces discrete, microscopic plaques in the WISH amnion cell line without addition of a semisolid overlay medium. This system can be used for the routine quantitative assay of this virus. The high degree of interferon sensitivity of WISH amnion cells, combined with the rapidity of VSV microplaque formation, contributed to the development of a human interferon assay which is quantitative, reproducible, easily performed and provides results within 24 hours (Green, Baron and Goode).

Interferon as a possible regulator of delayed hypersensitivity in the mouse. In some circumstances interferon inhibits expression of humoral and cellular immunity. The poly I.poly C, poly L-lysine, carboxymethyl cellulose complex induces interferon when applied topically in rabbits, mice and monkeys (Levy and Lvovsky). This complex was found to inhibit or decrease the contact sensitivity response to DNFB if applied before or within 4 hours after previously sensitized mice were reexposed to DNFB (Green, Williams and Levy).

Analysis of non-virion antigens of SV40, BK, and JC. The T, U, and TSTA (tumor specific transplantation antigens) of BK, JC, and SV40 were compared and analyzed. By complement fixation or immunofluorescence, the T-antigens induced by the 3 viruses were indistinguishable. In contrast, SV40 U antigen was synthesized in cells infected by BK and SV40, but not by JCV. In transplantation rejection tests, no protection was found in animals immunized with SV40 and subsequently challenged with BK or JC tumors, thereby showing that common TSTA was not shared by the 3 viruses (Takemoto, Mason; Law, NCI).

Complementation of tsA mutant of SV40 by BKV. tsA mutants of SV40 are defective in early functions, i.e., infected cells do not synthesize T-antigen or DNA at restrictive temperature. Cells co-infected with an SV40 tsA mutant and BKV produce full yields of SV40 tsA virus, proving that the early functions of BKV and SV40 are the same (Mason, Takemoto).

Biochemical analysis of the BKV, JCV, and SV40 genomes. In biochemical studies of the DNA's of the human papovaviruses and SV40 (in collaboration with Drs. Howley, Martin, and Khoury) the extent and localization of shared sequences have been determined. There is about 10% homology between JCV and SV40 and about 25% between JCV and BKV. The common sequences of JCV and SV40 are primarily in the "late" regions of the DNA, similar to what was previously observed between the DNA's of BKV and SV40 (Takemoto).

Tumor induction in hamsters by a nondefective Adenovirus 2-SV40 hybrid. Tumors containing both Adenovirus 2 and SV40 T antigens develop in hamsters injected as newborns with the UV inactivated nondefective Adenovirus 2-SV40 hybrid virus, $Ad2^{+}ND_4$, which carries 48% of the SV40 genome. As no viral induced neoplasms have been detected in hamsters injected with UV inactivated Adenovirus

2 or the Ad2^{+}ND$_{2}$ hybrid carrying 32% of the same region of the SV40 genome, the data show that a specific segment of SV40 DNA can alter the biological properties of Adenovirus 2 and convert it from a nononcogenic to an oncogenic human adenovirus (Lewis, Cook; Martin, LBV).

Reactions of sera from hamsters bearing tumors induced by Adenovirus 2 transformed cells with Adenovirus 2 polypeptides. Sera from hamsters bearing Adenovirus 2 tumors can be divided into 2 groups; those that react with Adenovirus 2 T antigens by complement-fixation and immunofluorescence and those that react with Adenovirus 2 T antigens only by immunofluorescence. Hamster sera which react with adenovirus 2 T antigen by both serologic procedures bind only to adenovirus 2 DNA binding protein and its breakdown products. Those sera which react only by immunofluorescence react with a different adenovirus polypeptide. These findings show that adenovirus T antigens are a complex group of polypeptides with different serologic specificities. Reaction by complement fixation is associated with only one group of these proteins (Chin, Maizel, NICHHD; Lewis).

Studies of clonally derived adenovirus 2 transformed hamster embryo lines. Lines of clonally derived adenovirus 2 transformed hamster embryo cells can be divided into 2 groups: those which induce tumors in newborns that grow progressively when transplanted in both newborns and weanling hamsters and those that induce tumors in newborns that grow progressively when transplanted into other newborns but either fail to grow or establish small neoplasms that regress when transplanted into weanlings. Studies on these cell lines indicate that the adenovirus 2 genome contributes factors which allow tumor cells induced by certain transformed lines to establish neoplasms in immunocompetent animals. These factors appear to be distinct from those which are responsible for transformation and could represent the expression of another part of the adenovirus 2 genome (Lewis).

Unique beaded virus of Entamoeba histolytica. Previous studies in LVD showed that E. histolytica carries two unique types of virus, icosahedral and filamentous. A third, morphologically distinct virus of E. histolytica has now been found. It replicates in the nucleus producing large, parallel arrays of beaded particles which individually are about 300 nm long and 18 nm in width. Isolated particles seen with negative staining are dominantly monomers of the above dimensions and consist of 14 beads. A dimer of twice the length and 28 beads is also seen (Mattern, Daniel and Kontonis, LVD; Diamond and Keister, LPD).

Amebal viruses and virulence of Entamoeba histolytica. Attempts have been made to determine if carriage of virus from a virulent culture increases the virulence of the recipient culture. Cultures of avirulent, axenically cultivated amebae have been substantially increased in virulence by subjecting the culture to a lytic amebal virus and cultivating the few surviving amebae. We believe that either the viral genome interacts with the amebal genome or, more likely, the virus selects a virus resistant and relatively virulent minority population from a mixed amebal culture (Mattern, LVD; Diamond and Keister, LPD).

Development of animal models for studying virulence of axenically cultivated

Entamoeba histolytica. To study the effect of infection on virulence of the amebae, it was necessary to try to improve the animal assays. The use of newborn hamsters has provided a practical model for studying intrahepatic amebiasis. Livers may be inoculated and observed for abscess formation through the skin. Within 7-10 days, as few as 20 virulent amebae produce liver lesions in about 25% of inoculated animals. Previously employed animal systems required about $2x10^6$ amebae to be inoculated intrahepatically into adult hamsters after laparotomy.

Newborn mice inoculated intracerebrally develop multiple, invasive amebic lesions resulting in brain abscess formation following inoculation of similar small doses of virulent amebae.

These two animal models are of potential utility in studying the pathogenicity of amebae, and the efficacy of drugs in treating extraintestinal amebiasis, which is a frequent and serious complication of human amebic dysentery (Mattern, LVD and Keister, LPD).

Administrative, Organization and Other Changes

During the past year there have been several changes in program emphasis.

The retirement of Dr. Sam Baron has resulted in a marked curtailment of LVD research on the biology of interferon. However, as indicated above, the various collaborative arrangements that Dr. Hilton Levy has established have functioned very well and have allowed him to make substantial contributions in the area of virus disease control by inducers of interferon. Extensive discussions were held concerning hiring Dr. Michael Oxman to replace Dr. Baron, in order to maintain a strong medically oriented in-house competence in antiviral chemotherapy. However, with the severe limitations on budget, space, and personnel during the time of these discussions, it became apparent that we could not provide him with adequate support and the negotiations were dropped.

An important new program area involves recombinant DNA research. Dr. Rowe was appointed to the NIH Program Advisory Committee on Recombinant DNA Research in July 1975; in addition to occupying a sizable proportion of his time, this activity has led to a commitment for conducting an intramural program in this area. This is to carry out, in collaboration with Dr. Malcolm Martin, LBV, extensive experiments in which the DNA of polyoma virus is inserted into prokaryotic vectors i.e., phage lambda and one or more E. coli plasmids; it will then be determined if mice can become infected with polyoma virus following infection with the vectors. Although we have been quite vociferous in trying to limit the extent to which the results of these studies should be extrapolated, it is clear that they will have a major impact on the degree of concern about the risks of recombinant DNA research. Because of this, we intend to ensure that this seemingly simple experiment covers a wide variety of experimental variables and that the experimental systems be characterized in great detail. The logistical requirements of this program, which requires P4-type physical containment, are being supplied by NCI, in the form of the mobile biohazard laboratory for the bacteriological work, and animal facilities at Fort Detrick, for the mouse studies. NCI Contract (NO1-CP-33288, "Development of Viral Diagnostic Tests and Map Testing Services"), with Microbiological Associates, Inc., under Drs. John C. Parker and Michael Collins, on which Dr. Rowe is a project officer, is also supplying a considerable amount of support for this program. Dr. Aurobindo Roy, presently a Visiting Fellow with Dr. Hoggan, will move to this program.

Dr. Lewis's program on non-defective adeno-SV40 hybrid viruses, essentially inactive since his initiation of a moratorium on work with these viruses outside of P3-type facilities 3 years ago, should be able to resume sometime during the coming year, when renovations in Bldg. 5 are complete.

Honors and Awards

Wallace P. Rowe

Harvey Lecture, New York, New York, March 18, 1976.
Recipient of National Academy of Sciences Selman A. Waksman Award in Microbiology, Washington, D. C., April 26, 1976.
Invited speaker, Johns Hopkins University School of Medicine, Department of Microbiology, Baltimore, Maryland, April 29, 1976.
Editor, ARCHIVES OF VIROLOGY
Consultant to the Board of Regents at University of Michigan, Ann Arbor, Michigan, May 12, 1976.
Appointed member of NIH Program Advisory Committee on Recombinant DNA Molecules.
Served on NIH Biohazard Committee.

Janet W. Hartley

Member, Virology and Cell Biology Advisory Committee, American Cancer Society.
Member, Virus Cancer Program Scientific Review Committee B (Solid Tumor Virus Segment).
Member, Editorial Board, JOURNAL OF THE NATIONAL CANCER INSTITUTE.

Andrew M. Lewis

Appointed member of the NIH Biohazard Committee.

M. David Hoggan

Convenor for the workshop on "Parvoviruses", Third International Congress of Virology, Madrid, Spain, 10-17 September 1975.
Participant, inauguration of the WHO-Centre for Collection and Evaluation of Data on Comparative Virology, Munich, Germany, October 1975.

Hilton B. Levy

Invited speaker Dr. Anfinsen's lab, NIAMDD, "Mechanism of Action of Interferon", November 1975.
Invited speaker, Department of Medicine, Hutzel Hospital, Detroit, Michigan, "Studies with Interferon", October 1975.
Invited speaker, University of Sienna, Sienna, Italy, "Therapy with Poly ICLC", April 1976.
Invited speaker, Wayne State University Medical School, Detroit, Michigan, "Therapy with Poly ICLC", April 1976.
Participant, Symposium, American Chemotherapy Society, Chicago, Illinois, August 1975.
Participant, Symposium, Stanford University, Stanford, California, September 1975.
Invited speaker, Dr. Hendler's lab, NHLI, "Studies on Interferon", February 1976.

Carl F. T. Mattern

Invited speaker, International Conference on Amebiasis, Mexico City, Mexico, October 27-29, 1975.

Kenneth H. Takemoto

Invited speaker, VIIth International Symposium on Comparative Research on Leukemia and Related Diseases, "Human Papovaviruses: Current Knowledge on their Biologic and Oncogenic Properties", Copenhagen, Denmark, October 1975.

Guest lecturer, Johns Hopkins University, "The Papovavirus Group".

Rex Risser

Session Chairman, RNA Tumor Virus Meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, May 29, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 AI 00010-13 LVD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunologic and Other Studies of Animal Leukemia Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Project Terminated

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00011-11 LVD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Small DNA Containing Viruses Belonging to the Family Parvoviridae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	M.D. Hoggan	Staff Scientist	LVD, NIAID
Other:	A. Roy	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

K. I. Berns, Johns Hopkins Univ. School of Med., Baltimore, Md.; N.L. Blacklow, Boston Univ. Med. School, Boston, Mass.; F. B. Johnson, Brigham Young Univ., Provo, Utah

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to elucidate the common biological, immunological, biophysical and biochemical characteristics of the various defective [adeno-associated viruses (AAV)]and nondefective (Rat virus, H-1, MVM, Haden, DNV, PPV Lu III and PLV) viruses belonging to the family Parvoviridae. To better understand their mode of replication and persistence in the latent state and investigate their role in various degenerative diseases.

Project Description:

Project is designed to compare the biological, immunological, biophysical and biochemical characteristics of various parvovirus populations in order to elucidate the basic mechanisms of parvovirus replication and/or long term latent persistence in their host cells. Efforts will continue to develop more efficient and sensitive techniques of parvovirus detection and identification. Such techniques will allow further study of the role these almost ubiqitous agents may play in many of the slow progressive diseases of unknown etiology found in man and animals. Parvoviruses or parvovirus-like agents have been tentatively identified as the cause of human hepatitis type A, and Aleutian mink disease. Because of the ability of these viruses to pass through the placenta, their extreme stability, and their propensity to persist in host in spite of high antibody levels, they remain good candidates to be the yet undiscovered etiological agents of lupus erythematosus, slow progressive CNS diseases and certain forms of arthritis.

Another area of parvovirus investigation is based on the observations that AAV decreases the oncogenic potential of Ad12 in newborn hamsters and that AAV infection of herpes simplex virus transformed hamster cells greatly reduces the tumorgenic capacity of such cells. Recent experiments have shown that infection not only reduces the number of tumors produced by a given number of transformed cell, but those tumors which are produced seldom if ever metastasize. An understanding of such a mechanism may prove to be of value not only in the prevention of certain malignancies but also in their treatment.

Early studies have shown that crude preparations of parvoviruses distributed as three distinct peaks after isopycnic centrifugation in CsCl. The main infectious peak, which contains particles with a full complement of single stranded DNA, is found at a density range of 1.385 to 1.43 gm/cm^3. These bands usually have physical particle to infectivity ratios (PP/IP) of less than 40, while a lighter peak banding at 1.30 to 1.36 gm/cm^3 contains only small amounts of DNA and has PP/IP ratios of 1000 or more. A third heavier band, with a relative specific density of greater than 1.45 gm/cm^3, contains infectious particles but they appear to have a slightly smaller diameter (about 12% smaller) and are more labile, tending to disintegrate after multiple bandings in CsCl. We have now shown that particles from each of these bands all contain one major and two minor polypeptides when analysed by SDS polyacrylamide electrophoresis, although the heavy (>1.45 gm/cm^3) particles appear to have a smaller proportion of the 2 minor polypeptides. Using immune-electronmicroscopy (IEM), we have found that antisera prepared against the two separate infectious bands show antigenic specificity for the particular band used in its preparation. Although there appears to be some cross reactivity, the amount of antibody which attaches is markedly different and a pattern of homologous agglutination is quite recognizable. The aggregates formed from light particles and homologous antibody are very tightly packed, while the particles in aggregates formed by heavy particles and their antibody appear to be separated by at least twice the distance as with the light particles. Whether the two distinct types of

antibody are found in natural infection and what effects, if any, they exert in vivo have yet to be investigated.

Our epidemiological studies of AAV from animals other than man and monkeys have been somewhat hampered because these viruses replicate to such low titers. We have recently found in collaboration with L. Cline of the Serology Unit that the use of the "Immune-adherence-hemmagglutination test" increases the sensitivity for measuring antibody by 2 to 8 fold, and of more importance, increases the sensitivity of measuring antigen by as much as a thousand fold. This test should allow us to do many more surveys, as well as increase our ability to recognize serological relatedness.

We and others have reported various latent carrier states in which various parvoviruses appear to be vertically transmitted from mother to offspring. These include the defective AAV's in man and monkeys and probably also include the AAV's of cattle, dogs, and chickens. Vertical transmission of the non-defective parvoviruses has been suggested for RV, H-1 and MVM of rodents, feline panleucopenia virus of cats, bovine parvovirus, and porcine parvovirus.

How the viruses are transmitted, and in what state the viral genome is in in the host cells, are not fully understood, but our studies on two model systems are providing some answers to these questions.

Using various hybridization techniques we have now shown that the latent AAV genome in our D-6 carrier cells is DNA which is integrated into the host cell DNA and that there are 3-5 viral genome equivalents per cell. We have shown that the virus produced upon challenge of these carrier cells with helper virus is biologically and immunologically identical to virus from extrinsically infected cells with helper. Further, the DNA of latent AAV is identical by restriction enzyme analysis to that of the extrinsic virus.

One difficulty in studying the biology and molecular biology of the AAV is the lack of a plaque technique whereby clonal lines of AAV can be produced for study. Using micro-titer plates we have developed a technique which allows us to "statistically clone" AAV in which only 5% of cultures will receive a single infectious unit of AAV. We have now prepared a number of AAV "clones" and are presently characterizing them.

We have noted that occasional lots of rat embryo fibroblast cells derived from colonies known to carry RV may appear normal with little or no evidence of virus contamination; however, these "normal" appearing cultures produce large quantities of RV when challenged with H-1 virus. We have attempted to develop a model carrier system in rat tail cell cultures for studying this helper effect. We have been successful in establishing a RV carrier cell line by adding RV antiserum to the tissue culture medium; these carrier cultures are relatively resistant to H-1 infection.

During our studies of the rodent parvoviruses we have isolated a large plaque virus which appears to be serologically distinct from a number of different RV strains but has the property of greatly inhibiting the production of RV

CF and HA antigen. Preliminary attempts to produce this virus without RV contamination have failed. Certain lots of MVM antisera cross react with this agent in HI test. Final identification is not yet complete.

Publications:

Johnson, F. B., Whitaker, C. W., and Hoggan, M. D.: Structural polypeptides of adenovirus-associated virus top component. Virology 65: 196-203, 1975.

Berns, K. I., Pinkerton, T. C., Thomas, G. F., and Hoggan, M. D.: Detection of adeno-associated virus (AAV)-specific nucleotide sequences in DNA isolated from latently infected Detroit-6 cells. Virology 68: 556-560, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00012-14 LVD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Mouse Leukemia Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dr. Janet W. Hartley, LVD, NIAID
Dr. Wallace P. Rowe, LVD, NIAID
Dr. Sisir K. Chattopadhyay, DCT, NCI
Dr. Stephen P. Staal, LID, NCI
Dr. Akinori Ishimoto, VO, NCI
Dr. Rex Risser, LVD, NIAID
Dr. Eli Canaani, LVD, NIAID (from Oct. 1975)

COOPERATING UNITS (if any) M. Potter, LCBGY, NCI; T. Chused, OMS, NIDR; H. Morse, LMI, NIAID; T. Bremner, Howard Univ.; J. Parker, Micro. Assoc., Inc.; P. Pitha, Johns Hopkins Univ., E. Stockert, Sloan Kettering Instit. for Ca. Res.; F. Ruddle, Yale; D. Bailey, Jackson Labs.; E. Boyse, Sloan Kettering; H. Ikeda, Sloan Ketter.

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14.7	5.4	9.3

SUMMARY OF WORK (200 words or less - underline keywords)

A combination of virologic, genetic, and biochemical approaches is being used to study the natural history of murine leukemia virus (MuLV) infection. Following the plan of previous genetic studies, which delineated two virus-inducing loci in the high ecotropic virus, high leukemia AKR strain of mice, mouse strains selected for interesting biological characteristics (e.g., NZB, a high producer of xenotropic virus; B10.Br/Li, which is chemically inducible for both N- and B-tropic ecotropic virus; RF/J and PL, high ecotropic virus strains) are being appropriately mated with phenotypically defined mouse strains and the hybrids tested for MuLV production and/or virus inducible by IUDR treatment. The several studies underway are expected to answer the question of whether a single gene region can code for more than one virus genotype, may permit mapping of virus loci in additional mouse strains, and could yield information about the relation of xenotropic virus to spontaneous disease. Nucleic acid hybridization techniques are being used in the genetic studies and to characterize a variety of mouse strains for unique and common viral DNA sequences. Methods for producing and quantitating phenotypic mixtures of ecotropic and xenotropic MuLV have been developed. Two apparently new host range variants of MuLV have been detected in wild mice, and studies of the pathogenesis of wild mouse ecotropic and amphotropic viruses are in progress.

Project Description:

Objectives: To utilize sensitive procedures for genome detection and isolation and quantitation of infectious virus in studying the natural history of murine leukemia virus (MuLV) infection.

Methods Employed: Tissue culture procedures, using plaque and transformation assays; tests for antigen induction by complement fixation and fluorescent antibody procedures; reverse transcriptase assays. Mouse inbreeding, to select for specific virus-inducing or virus-related genes. Starch-gel electrophoresis for assays for marker isoenzyme loci. Nucleic acid hybridization techniques.

Major Findings:

Biochemical studies of MuLV infection: Studies of unique and multiple copy viral sequences in mouse cellular DNA (detected by hybridization with ^{3}H-DNA probes prepared from AKR viral RNA) have been extended by testing of additional strains of inbred mice and a small number of wild rodent species, and by testing with probes prepared from xenotropic MuLV. These studies are designed to analyze further the correlations between type of viral genetic information detected and the virology of the mouse strain, to examine the nature of the multiple copy viral sequences found in the mouse genome, and to study the evolution of the viral genome. In collaboration with Frank Ruddle and Christine Kozak, Yale University, DNA preparations from somatic cell hybrids between hamster and mouse, with defined chromosomal segments from the A strain mouse, are being tested by nucleic acid hybridization in an attempt to detect viral sequences at specific chromosomal sites.

Work on demonstrating the circularity of the MSV provirus, begun by Dr. Canaani in Peter Duesberg's laboratory is being completed here.

Genetics of ecotropic and xenotropic virus induction in B10.Br/Li mice:

Studies of the genetic transmission of MuLV in B10.Br/Li mice (an Fv-1^{b} strain showing relatively high expression of B-tropic virus in adult mice and inducibility of both N- and B-tropic virus from embryo or tail tissue cultures) have been expanded to include matings with both Fv-1^{n} and Fv-1^{b} partners and testing for xenotropic virus expression. Although definitive conclusions are not yet possible, these matings are designed to determine whether induction of different host range types of virus can occur from a single region of the mouse genome.

Methods have been developed for induction and detection of virus in cell cultures prepared from tail tissue, permitting virologic characterization of large numbers of individual animals without sacrificing them or adversely affecting their breeding potential.

Murine leukemia viruses in wild mice: In addition to the previously described N-tropic ecotropic viruses and amphotropic viruses (Fv-1^{n} mouse and heterologous

species tropism), two additional types of MuLV have been detected in wild mice from the Lake Casitas, California trapping area. Tests of IUDR-induced embryo cultures have yielded two mouse-tropic isolates whose host range appears to be limited to the Sc-1 line of wild mouse embryo, a clonal line which lacks the Fv-1 gene restriction seen in laboratory mice. Also, two isolates have been obtained which are very similar to xenotropic MuLV. Tests are in progress to obtain limiting dilution cloned virus and to characterize the new isolates by neutralization and interference patterns.

In collaborative studies with John C. Parker and Sara Hourihan, Microbiological Associates, mouse-tropic and amphotropic MuLV isolates from wild mice have been tested for pathogenicity by intracerebral inoculation of newborn NIH Swiss mice. Results to date indicate that the lower limb paralytic disease described by Gardner et al. can be induced by the mouse-tropic but not by the amphotropic isolates, while both types of virus can induce lymphoma.

Detection of xenotropic viruses: Studies on detection of xenotropic virus in NZB and NZW mice and their genetic crosses have been done in collaboration with Thomas Chused and Herbert Morse using infectious center plating of mitomycin C-treated lymphoid cells on mink cells (tested by immunofluorescence) or on the mink S+L- cell described by Paul Peebles. These studies have revealed 1) evidence for a single gene for production of xenotropic virus in NZB (in confirmation of earlier studies by Stephenson and Aaronson); 2) a higher frequency of infectious virus expression in cells derived from NZB bone marrow and spleen, as opposed to thymus and lymph node; 3) expression of infectious virus by both T and B cells derived from NZB spleen.

Elisabeth Stockert and Lloyd Old have found that thymus cell surface expression of several MuLV-related antigens (G_{IX}, GCSA, gp 70, and p 30) is markedly increased in preleukemic 6 month old AKR mice, in comparison to 2 month old mice. Tests for infectious virus production by thymus cells obtained from mice hemithymectomized and scored for these antigens by Dr. Stockert have revealed no significant difference in ecotropic virus expression between mice with high and low antigen levels, but a marked increase in xenotropic virus or possibly phenotypically mixed mouse and xenotropic virus expression in the high antigen level mice.

Genetic control of susceptibility to Abelson murine leukemia virus (MuLV-A):

BALB/c (C) mice are highly susceptible to B- cell lymphoma induction by MuLV-A, a complex of defective transforming virus and helper leukemia virus. Susceptibility is independent of age at time of inoculation, 3 month old mice being essentially as susceptible as sucklings. In contrast, C57Bl/6 (B6) mice, although sensitive to lymphoma induction at 1 week of age, are highly resistant by 6 to 8 weeks of age. Studies of the virology of infected mice and of the susceptibility of CxB6F_1 mice and the 7 recombinant inbred CxB6 lines established by D. Bailey (Jackson Laboratory) indicate that B6 mice carry a major recessive gene providing resistance to MuLV-A lymphoma induction and that the mechanism of resistance involves control of the replication of the transforming component or cells producing it. No correlation has been

detected between Fv-1, Fv-2, or sensitivity to pristane plasmacytoma induction and MuLV-A susceptibility.

Studies of MuLV phenotypic mixtures: Techniques for production and quantitation of phenotypic mixtures of MuLV's with different host ranges have been developed by Dr. Ishimoto. Using a modified infectious center assay, he has determined the relative concentrations of ecotropic and xenotropic virus and reciprocal phenotypically mixed particles in various virus preparations. Tests of the host range of xenotropic virus pseudotypes of N-, B-, and NB-tropic MuLV's indicate that host cell restriction is correlated with the host range of the ecotropic component, suggesting a role for the Fv-1 gene in this system.

Effect of interferon on MuLV replication: In studies carried out by Paula Pitha in collaboration with us and Michael Oxman it was found that interferon inhibits replication of exogenous virus, activation of endogenous virus by IUDR, and production of virus by chronically infected cells. The effect was not to suppress infection or induction completely, but to delay appearance of infectious virus; rapid recovery of virus production occurred in chronically infected cells after removal of interferon. Interferon appears to inhibit one or more late steps in viral replication, occurring after the expression of viral gs antigen and prior to virus assembly.

Significance to Biomedical Research and the Program of the Institute:

Unravelling the complexity of naturally occurring MuLV populations, exemplified by the ecotropic, xenotropic, and amphotropic classes and the possible phenotypic and genotypic recombinants which could be generated, is a necessary part of studies aimed at defining the role of these viruses in spontaneous tumorigenesis. The mouse model appears to provide the most fruitful approach to delineating the biological and molecular biological processes involved in cancer. The combined approach of virologic, genetic, and biochemical analysis provides enhanced sensitivity and precision in eventual correlation of presence and expression of viral information with disease.

Proposed Course: Attempts will continue to localize viral integration sites in various strains of mice and to determine whether genetic information for different host range types of virus can occur in a single region of the genome. Various MuLV viral DNA probes will be used in comparing viral sequences in strains of mice with differing expressions of MuLV information; tests of DNA from other rodent species for MuLV sequences will be continued. Using somatic cell hybrids, attempts will be made to determine whether the viral sequences common to all mice (i.e., the multiple copy sequences detectable by nucleic acid hybridization) occur in large clusters or scattered through the genome. Somatic cell hybrids may also be used in attempts to localize AKV-2, the unmapped virus-inducing locus in AKR mice, and to determine whether it is allelic with analogous sites in other mouse strains.

Characterization of the MuLV population in laboratory and wild mice will concentrate on classification of new strains, tests for viral interactions in

attempts to clarify the origin of the multiple virus types detected, and pathogenesis studies. Wild mice from other geographic areas will be tested for amphotropic virus expression and genetic information.

Studies on the genetics of xenotropic virus transmission and spontaneous disease in NZB hybrids with NZW and inbred NIH Swiss will be continued.

Continued study of the genetics of susceptibility of inbred mice to Abelson virus will include attempts to map the locus in C57Bl/6 which confers resistance.

Work on the structure of the MuLV provirus will begin with characterization of naturally occurring MuLV strains of restriction enzyme patterns.

Publications:

Hartley, J.W. and Rowe, W.P.: Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology, 65: 128-134, 1975.

Hartley, J.W. and Rowe, W.P.: Naturally occurring murine leukemia viruses in wild mice: Characterization of a new "amphotropic" class. J. of Virology, 1976. In press.

Ikeda, H., Rowe, W.P., Boyse, E.A., Stockert, E., Sato, H., and Jacobs, S.: Relationship of infectious murine leukemia virus and virus-related antigens in genetic crosses between AKR and the Fv-1 compatible strain C57L. J. Exp. Med., 143: 32-46, 1976.

Pincus, T., Hartley, J.W., and Rowe, W.P.: A major genetic locus affecting resistance to infection with murine leukemia viruses. VI. Dose-response relationships in Fv-1 sensitive and resistant cell cultures. Virology, 65: 333-342, 1975.

Pitha, P.M., Rowe, W.P., and Oxman, M.N.: Effect of interferon on exogenous, endogenous, and chronic murine leukemia virus infection. Virology, 1976. In press.

Pitha, P.M. and Rowe, W.P.: Effect of interferon on exogenous, endogenous, and chronic murine leukemia virus infection. JNCI Monograph-Second Conference on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, 1976. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00013-14 LVD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Viral Antigens in Virus-induced Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Andrew M. Lewis, Jr.	LVD NIAID	
Other:	James L. Cook	Research Associate	LVD NIAID

COOPERATING UNITS (if any) Malcolm Martin (LBV/NIAID); Arthur Levine & Cephas Patch (Pediatric Oncology Br., NCI); William Chin & Jacob Maizel (NICHHD); Michael Oxman & Myron Levine (Harvard U.); Satvir Tevethia (Tufts U.); Lenneart Philipson (U. Uppsala)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.75	2	2.75

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to use human adenoviruses and adenovirus-SV40 hybrid viruses as tools to study the genetics of DNA tumor viruses; to define the role of viral genes and viral antigens in viral induced tumors and to study the biology of adenovirus-SV40 hybrid viruses. Due to the lack of proper facilities to pursue studies with nondefective Adenovirus 2 SV40 hybrids during the past 3 years, the major emphasis of the project has gradually shifted to a study of the oncogenic properties of human Adenovirus type 2.

Project Description

Major Findings - Studies on nondefective adenovirus - SV40 hybrid viruses: The properties of the five nondefective adenovirus 2-SV40 hybrids which we have isolated and characterized were described in the 1972-1974 project reports. Due to a lack of appropriate containment facilities for work with these agents, studies during the past year were confined to continuing an evaluation of the tumor inducing capacity of hybrids rendered noninfectious by exposure to UV light. We have observed the development of tumors in 10% of hamsters injected with UV inactivated $Ad2^{+}ND_{4}$ and B55 hybrids. Animals carrying these tumors developed antibody to both Ad2 and SV40 T antigens.

Tissue culture transformation by UV-inactivated Ad2 and nondefective hybrids: Our previous studies had indicated that, in assays with UV-killed virus, the $Ad2^{+}ND_{4}$ and B55 hybrids were somewhat more efficient in transforming hamster cells than either nonhybrid Ad2 or the $Ad2^{+}ND_{2}$ hybrid. Each of the nondefective hybrids was more efficient in transforming rat cells than was nonhybrid Ad2. The virus stocks used in these experiments were prepared prior to 1973 in diverse lots of primary human and monkey renal cells. To assess the importance of cells used to prepare virus stocks for use in transformation assays, stocks of Ad2, $Ad2^{+}ND_{1}$, $Ad2^{+}ND_{2}$, $Ad2^{+}ND_{4}$ and the B55 hybrid were prepared in the same lot of Vero cells in the NCI collaborative containment module in Building 41. These stocks were then used to transform a single lot of rat embryo cells. In this experiment there were no significant differences in the efficiency of transformation between Ad2 and the nondefective hybrids. Although we cannot be sure that the genomes of the nondefective hybrids were unaltered during passage in Vero cells, these experiments suggest that factors other than the viral genome can influence the results of comparative transformation assays. Until appropriate containment facilities are available for a resumption of work with infectious hybrid viruses we will not be able to continue our investigation of this problem.

Induction of tumors in hamsters with Ad2 transformed cells: When cells appear morphologically transformed in tissue culture, it is important to determine whether they can induce tumors in appropriate hosts. To prove that the foci being scored in the Ad2 transformation assays represent colonies of malignant cells, cell lines have been established from a number of colonies and injected into newborn hamsters. Of the 10 lines of Ad2 transformed inbred LSH hamster embryo cells examined, all induce fatal tumors when injected into either newborn LSH or NIH hamsters. When tumors induced in newborn animals are transplanted into 21 day old LSH or NIH hamsters, however, only tumors induced by 2 of the 10 cell lines will produce progressively growing neoplasms. When transplanted into 21 day old animals, tumors from newborns induced by 6 of the 10 cell lines generally produce no tumors or small tumors (20-25 mm in diameter) which regress over a 4-8 week period. Tumor regression occurs in 21 day old hamsters even after 10-17 passages of the tumor line in newborn hamsters. Studies of newborn tumors induced by these 6 transformed cell lines show that varying the tumor producing dose injected, the route of inoculation, or inoculation schedules, do not alter the inability of these tumor lines to induce progressively growing neoplasms in 21 day old hamsters.

Weanling hamsters carrying the two progressively growing tumor lines developed different patterns of antibody to early adenovirus antigens. Animals carrying tumors induced by the Ad2HE$_1$ line developed antibodies to early Ad2 T antigen which were detectable by both immunofluorescence and complement-fixation. Weanling hamsters carrying progressively enlarging tumors induced by the Ad2 HE$_9$ line developed only immunofluorescent detectable Ad2 T antibodies. Weanling animals carrying the few progressively enlarging tumors induced by the remaining Ad2 transformed lines after the inoculation of 25 times the usual dose of tumor cells also develop Ad2 T antibodies detectable only by immunofluorescence.

One of the simplest explanations for the regression of Ad2 induced tumors in weanling hamsters is that the regressing tumors induce larger quantities of Ad2 transplantation antigen then the progressively growing tumors, thus provoking a strong rejection response from their immunocompetent host. Studies are underway to assess Ad2 transplantation antigen induction by these cell lines. Preliminary results indicate that there is no difference in the ability of the two types of Ad2 cell lines (those inducing progressing and those inducing regressing tumors) to induce Ad2 transplantation rejection antigens in hamsters. Studies confirming these findings are underway.

Since the Ad2 transformed cell lines being studied are clonally derived, the data thus far imply that the Ad2 genome contributes factors which allow tumor cells induced by certain lines to establish neoplasms in immunocompetent animals. Furthermore, the data at this time suggest that these factors are distinct from viral induced transformation and could represent the expression of another part of the Ad2 genome.

Effects of neonatal thymectomy on tumor induction by Ad2 transformed hamster embryo cells: The tumor lines of Ad2 transformed hamster cells which will not grow in weanling hamsters will cause progressively growing tumors in weanlings which had been thymectomized within 24-48 hours of birth. This observation suggests the importance of the thymus-derived peripheral "T" lymphocyte in mediating the rejection of the Ad2 tumor lines which will not cause tumors in immunocompetent weanlings. The peripheral T lymphocyte is thought to be the cell primarily responsible for graft rejection; thus, the permissive effect of thymectomy on tumor growth could be explained by a quantitative difference in transplantation antigen present on the cells of the rejected lines when compared to the cells of the line causing progressively growing tumors in weanlings. However, our preliminary results suggest that there is no significant difference between the quantity of transplantation antigen manifested by a "regressor" line when compared to a "progressor" line.

Since there was some difference in susceptibility of batches of thymectomized hamsters to tumor challenge, a method was needed to determine if some animals were only partially thymectomized and thereby resistant to tumor transplantation. We have used concanavalin A (Con A), a mitogen which selectively

stimulates T lymphocytes to undergo blastic transformation, in an in vitro system as an indicator of the level of T cell function in LSH hamsters. Using immunocompetent animals, optimum culture conditions have been established for lymphocytes derived from both whole blood and spleens. We have also shown that LSH hamsters during the first week of life have significantly lower Con A responses, hence lower T lymphocyte function, than do weanlings. This finding is compatible with the hypothesis that maturation of T lymphocyte function is important in tumor rejection.

Reaction of sera from Ad2 tumor bearing hamster with early Ad2 polypeptides: In experiments with Drs. Chin and Maizel we are studying sera from weanling hamsters carrying tumors induced by various Ad2 transformed hamster cells for their reactivity with Ad2 polypeptides separated by SDS polyacrylamide gel electrophoresis. Preliminary results indicate that hamster sera which react with Ad2 T antigens by complement fixation react with 72K (Ad2 DNA binding protein), 70K, 60K and 45-48K polypeptides (these polypeptides seem to represent breakdown products of DNA binding proteins). Sera from those tumor bearing hamsters which react with Ad2 T antigens only by immunofluorescence only react with a 58-60K polypeptide (different from DNA binding protein or its products). By comparing the reactivities of sera from hamsters carrying tumors induced by the various types of Ad2 transformed cell induced tumor lines, we hope to associate specific Ad2 polypeptides with specific biologic functions of the Ad2 genome as expressed in the transformed cell.

Significance to Biomedical Research and Proposed Course of the Project: Due to conflicting results we are unable to interpret our data about the influence of the SV40 genome on the efficiency of in vitro transformation of hamster and rat cells by the nondefective Ad2-SV40 hybrids. Tumor induction studies with UV inactivated viruses still suggest, however, that the acquisition of that portion of the SV40 genome lying between the end of the SV40 segment in $Ad2^{+}ND_2$ (at .32 SV40 units) and the end of the ND_4/B55 segment (at .48 SV40 units) conveys upon Ad2 the ability to produce tumors when injected into newborn hamsters. This is the region of the SV40 genome which is associated with the induction of SV40 T antigen. Studies verifying these conclusions will be completed in the next few months.

The morphologic alteration of cultured cells by oncogenic viruses is frequently referred to as "malignant transformation". This concept implies that the altered (transformed) cells possess the capacity to induce neoplastic disease in appropriate hosts. Thus transformation of cells in tissue culture is equated with in vitro carcinogenesis. However, studies on the tumor inducing capacity of polyoma transformed hamster cells, SV40 transformed mouse cells and Ad 1, 2, 5 transformed rat cells demonstrate that the association between in vitro transformation and the ability of the transformed cells to induce neoplasms when injected into appropriate hosts is inconstant and complex.

Our studies show that Ad2 transformed hamster cells consistently produce tumors in newborn hamsters. The majority of newborn tumors induced by these lines fail to induce neoplasms when transplanted to immunocompetent weanling animals. Removal of the hamster's thymus shortly after birth alters the immune system and permits regressing tumor lines to establish neoplasms in a thymic host. To explain the role of host immunity in the development of neoplastic disease in animals receiving cells transformed by DNA viruses two possibilities must be considered. First, certain transformed cell lines could produce excessive amounts of transplantation rejection antigens which immunize the host before a critical tumor mass is obtained and thus set the stage for tumor rejection. Second, the viral genome, in addition to transforming the hamster cells and inducing TSTA, could render certain lines more resistant to normal host cellular immunity. Our preliminary data suggest that for Ad2 transformed hamster cells the second explanation is correct. Studies are underway to test this conclusion.

Several studies have demonstrated that early Ad2 polypeptides can be characterized by high resolution SDS polyacrylamide gel electrophoresis. Knowing that reactivities of sera from hamsters carrying tumors induced by different Ad2 transformed lines vary, we plan to characterize these patterns and determine whether progressive tumor growth can be associated with the presence or absence of certain early polypeptides.

In addition, experiments are underway to determine which Ad2 DNA sequences are represented in the Ad2 transformed hamster lines. By combining the biological and molecular biological studies with an evaluation of cellular immunity in hamsters, we should be able to better understand the role of early antigens and polypeptides in the induction and regression of tumors produced by Ad2 transformed cells.

Publications:

Siegel, S.E., Patch, C.T., Lewis, A.M., Jr., and Levine, A.S.: Simian virus 40 DNA replication, transcription, and antigen induction during infection with two adenovirus 2-SV40 hybrids that contain the entire SV40 genome. J. of Virol., 16: 43-52, 1975.

Tevethia, S.S. and Lewis, A.M., Jr.: Two adenovirus type 2 - simian virus 40 hybrid populations which contain the entire SV40 genome differ in their ability to induce SV40 transplantation immunity in hamsters but not in mice. J. of Virol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00014-09 LVD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Interferon and Interferon Inducers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Virology Section

INSTITUTE AND LOCATION
NIH, NIAID, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

Project combined with and reported under Project #Z01 AI 00019-02 LVD

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 A1 00015-10 LVD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure, Properties and Replication of Viruses of Pathogenic Protozoa and Mammals.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Carl F. T. Mattern, LVD, NIAID
Wendell A. Daniel, LVD, NIAID
Anna D. Kontonis, LVD, NIAID
Katherine Cook Jaouni, LVD, NIAID

COOPERATING UNITS (if any)

Dr. Louis S. Diamond, LPD, NIAID
Dr. Shigeko Nomura, VLL, NCI

LAB/BRANCH
Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION
Cellular Virology Section

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4-1/2	3-1/2	1

SUMMARY OF WORK (200 words or less - underline keywords)

The morphogenesis of a unique amebal virus of Entamoeba histolytica (Rahman strain) has been studied by electron microscopy. Arrays of parallel packed beaded viruses 300 nm long assemble in the nucleoplasm. The virus isolated in CsCl gradients exists as a monomer of 14 beads or sometimes as a dimer of 28 beads and 600 nm long.

Two animal models for studying the virulence of E. histolytica have been developed. Brains of newborn mice are susceptible to amebae inoculated intracerebrally. Amebae produce a fatal invasive disease with multiple abscesses.

The newborn hamster liver is extremely susceptible to E. histolytica, which can be inoculated intrahepatically through the abdominal wall without laparotomy. Progress of disease can also be followed by observing the liver in situ. The disease consists of typical amebic abscesses containing trophozoites.

Embryonated eggs and mammalian tissue cultured cells have been found less promising than newborn animals in studying virulence of E. histolytica.

Avirulent amebae have been rendered moderately virulent by establishing a culture of survivors of a lytic virus infection. We do not know whether this represents simply the selection of both a virus resistant and virulent population or whether there is a more complex interaction of virus and amebae.

Project Description:

The major emphasis of this project is currently being placed on the study of viruses of Entamoeba histolytica. Two aspects of this study have shown considerable development in the past year: the purification of the beaded virus and the virulence of Entamoeba histolytica. These studies were undertaken in collaboration with Dr. Diamond's group in LPD.

A third amebal virus. A unique beaded viral agent, has been investigated in some detail. The intranuclear replication of the virus was studied by electron microscopy. It assembles as large ordered arrays of parallel packed, beaded rods about 300 nm in length. Isolated particles seen by negative staining usually are 300 nm long and consist of 14 beads. Only occasional shorter and presumably broken fragments of random length are seen. A second class of particle commonly seen is obviously a dimer of about 600 nm length and consisting of exactly 28 beads.

In order to study the virulence of E. histolytica and a possible role of virus therein, it was first necessary to develop animals systems in which the virulence of axenically cultivated amebae could be evaluated conveniently. In collaboration with D. B. Keister (LPD) two such animal models have been developed; the work over the past 6 months is being prepared for publication.

Amebic lesions in the brain. Amebic abscesses can be produced in a few animals by as few as 20 virulent amebae injected into the brain of the newborn mouse. Mice 5 or 6 weeks of age are increasingly resistant to virulent amebae inoculated intracerebrally (IC). The amebae invade the brain tissue with round cell infiltration, multiple abscesses formation, amebal replication and death of the mouse within 3-20 or more days depending on the number of amebae injected. Some axenic amebal strains are totally avirulent, others show moderate virulence.

Newborn mice inoculated with avirulent amebae or older mice inoculated with virulent amebae often develop hydrocephalus 2 to 5 weeks after inoculation. The pathology of this disease is currently under study.

Hepatic amebic abscesses. Heretofore the only available animal in which liver disease could be produced by axenic amebae was the hamster, which required from 2x10 to 10x10 amebae to be inoculated intrahepatically at laparotomy. Large scale studies are impossible with this animal system. Newborn hamsters (but not so newborn mice) were found by us to be extremely susceptible to axenically grown E. histolytica inoculated intrahepatically (IH) directly through the abdominal wall. Not only can liver be

readily visualised for inoculation, but these hepatic lesions presenting on the anterior surface of the liver can be easily seen as early as 4 days after inoculation. Animals were sacrificed on the 7th to 10th day and autopsied. Liver lesions were scored by number and size. As few as 20 virulent amebae produced hepatic abscesses in 10 days and the percent of infected animals increased with dosage to 100% of animals infected with 2000 virulent amebae. Our studies to date involve large numbers of newborn hamsters and show the virulence assay to be highly stable and reproducible. Both of these animal systems have considerable potential usefulness in the testing of drugs for their effectiveness against extraintestinal amebiasis.

Additional virulence models have been investigated. K. Jaouni has investigated various routes of inoculation of embryonated eggs with axenically grown amebae and Anna Kontonis has employed mammalian tissue culture systems. Both show some promise, but neither appears as reproducible and practical as the animal model and will not be developed further at this time.

Viruses and virulence of E. histolytica. Employing the animal systems described above some preliminary data of interest has been obtained in collaboration with D. B. Keister (LVD). HK-9 are avirulent either intracerebrally in the mouse or intrahepatically in the hamster. HK-9 amebae which were infected with viruses either from HM-1 (virulent) or 301 (moderately virulent) amebae were initially lysed, but the few survivors were cultivated and eventually healthy cultures were established. Such survivor cultures were found to produce the appropriate new virus and were resistant to superinfection. These survivor cultures were both considerably more virulent intrahepatically in the hamster than the parental HK-9 strain. The virus of HM-1 produced a survivor culture of HK-9 that was not nearly as virulent as the HM-1 amebae. The virus of 301 amebae did, however, produce an HK-9 survivor culture that was more virulent than either the parental HK-9 or the 301 virus donor strains. At this time we do not know whether the virus infection simply selects both for resistance and virulence from a mixed population of wild-type HK-9 amebae or whether there is lysogeny and the integrated viral genome alters the amebal virulence. The former seems more probable on the basis of other available data. Of 4 clones of HK-9 amebae assayed for hepatic virulence, 2 were avirulent, one was slightly virulent and the fourth was of about the same moderate virulence as the 2 above mentioned survivor cultures. It is nonetheless of interest that we are able to either select for virulence or modify amebal virulence with the viruses of E. histolytica.

We are continuing to study a large number of clones of different amebal strains and a variety of survivor cultures in an attempt to further elucidate the nature of the virulence alteration.

Publications:

Paran, N., Mattern, C.F.T. and Henkin, R.I. Ultrastructure of the taste bud of the human fungiform papilla. Cell and Tissue Res. 161:1-10, 1975.

Mattern, C.F.T. and Paran, N. Observations on the origin of the dense extracellular pore substance of the bovine taste bud. Trans. Amer. Microsc. Soc. 95:56-62, 1976.

Nomura, S., Fischinger, P.J., Hartley, J.W. and Mattern, C.F.T. Induction of a novel transforming virus (MSV-2) from sarcoma-positive leukemia-negative 3T3FL mouse cells. Virology 69: 635-646, 1976.

Diamond, L.S. and Mattern, C.F.T. Viruses of protozoa. Adv. Virus Res. 20, 1976.

Mattern, C.F.T. Symmetry in virus architecture in Molecular Biology of Animal Viruses. D. Nayak, Ed. Marcel Dekker, N.Y. 1976 in press.

Pertel, R., Paran, N. and Mattern, C.F.T. Ultrastructural localication of cholinesterase associated with the anterior structures of Caenorhabditis elegans. Exp. Parasit., April, 1976.

Diamond, L.S., Mattern, C.F.T., Bartgis, I.L., Daniel, W.A., Keister, D.B. Viruses of Entamoeba histolytica VI. A study of host range. Proc. Intern. Conf. Amebiasis. Oct., 1975, in press.

Mattern, C.F.T., Diamond, L.S., Daniel, W.A., and Keister, D.B. Unusual variation of "Normal cytoplasmic bars of Entamoeba. Proc. Intern. Conf. Amebiasis, October, 1975, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00017-09 LVD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Host Defenses Against Viral Infection: Interferon and Immunity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Virology Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

Project combined with and reported under Project #Z01 AI 00019-02 LVD

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 A1 00018-10 LVD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biological and Biochemical Characterization of Human Papovaviruses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dr. Kenneth K. Takemoto, LVD, NIAID
Dr. David Mason, LVD, NIAID
Dr. Sheila Bond, LVD, NIAID
Dr. Tatsuo Miyamura, LVD (Guest Worker, Japan)
Dr. Malcolm Martin, LBV, NIAID
Dr. Peter Howley, NCI

COOPERATING UNITS (if any)

Dr. Charles Holmberg, Univ. Calif., Davis
Dr. David Gribble, Univ. Calif., Davis

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Virology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

SUMMARY OF WORK (200 words or less - underline keywords)

The emphasis of research in this laboratory continues to be directed toward increasing our knowledge on the biology and biochemistry of the newly recognized human papovaviruses, BK and JC. Based on studies carried out in collaboration with Drs. Howley, Khoury, and Martin (LBV), a physical map of the BKV genome has been constructed. In addition, some information on JCV genome has been obtained and the extent and localization of common sequences between BKV, JCV, and the simian virus 40 (SV40) is now known.

Since some of the early functions and gene products (T-antigens) appear to be shared between the human and simian papovaviruses, other studies were under taken to study further the known early functions of the papovaviruses. Results of these investigations have shown that: (1) virus-specific transplantation antigens are not shared by the 3 viruses, (2) U-antigen is induced by SV40 and BKV, but not by JCV, (3) T-antigens induced by the 3 viruses are indistinguishable by fluorescent antibody or complement-fixation tests. Complementation studies have shown that BKV can provide the early functions defective at restrictive temperatures in tsA mutants of SV40, thus providing additional information on the relationship between the 2 viruses.

Project Description:

Objectives: To characterize as completely as possible, the biology and biochemistry of the human papovaviruses, JCV and BKV. These studies include in vitro and in vivo oncogenic potential, host range for productive and abortive infections, and biochemical studies on viral genomes to determine relatedness of their DNAs.

Methods employed: Standard virologic and serologic methods are employed. For virus growth, human embryonic fibroblast or epithelial cultures are used for virus production and plaque assays of BKV; human fetal brain cells are grown as primary cultures for JCV virus production. Complement-fixation and immunofluorescent methods are used for T-antigen studies.

Major findings:

Biochemical studies: Analysis of the DNA of BKV and 3 isolates of related viruses from Wiskott-Aldrich patients by hybridization techniques have revealed no differences. However, by restriction endonuclease cleavage, one of them, BKV(MM), showed only 3 fragments as contrasted to 4 fragments found in all other isolates. Physical maps of the BKV and MMV genomes were constructed, and these were oriented in relationship to the map of SV40 DNA.

Although JCV studies continue to be severely hampered by the unusually restricted host range of this virus (its replication is limited to human fetal brain cells, exclusively), we were nevertheless able to obtain labelled viral DNA to perform preliminary studies of the relationship of this virus to BKV and SV40. DNA hybridization experiments have shown that there is about 10% homology between JCV and SV40 and about 25% between JCV and BKV. The shared sequences with SV40 appear to be primarily in those regions of the DNA responsible for late functions. This is similar to what was previously observed with BKV.

Comparison of SV40 functions with BKV and JCV: Three of the early non-virion antigens induced by SV40 in lytic or in transformed cells have been investigated and compared with BKV and JCV. The T-antigens are indistinguishable as measured by fluorescent antibody or complement-fixation tests. U-antigen, a poorly understood antigen induced by SV40, is also synthesized in cells infected by BKV; BKV transformed cells also show weak reactivity with anti-U serum. By contrast, U antigen was not detected in cells productively infected or transformed by JCV.

In transplantation rejection studies, hamsters immunized with SV40 virus did not show any resistance to challenge with BK or JC

tumors. Thus, common tumor specific transplantation antigens (TSTA) are not shared by the 3 viruses.

Complementation studies with BKV and temperature sensitive mutants of SV40 have been completed. Group A ts mutants, which are unable to carry out early functions at restrictive temperatures, are complemented by BKV, and full yields of SV40 are obtained. Group B mutants are defective in late functions, i.e., assembly and maturation; these are not complemented by BKV, which is also defective in monkey cells and thus cannot supply the missing late functions.

Transformation: Using the calcium phosphate co-precipitation technique, hamster cells were successfully transformed with BKV DNA. These cells possessed all the properties of transformed cells. An unusual finding in these studies was the production in tumor-bearing hamsters of anti-nuclear antibody (ANA) which reacted with normal cells from a variety of species. Serial transplants of BK tumor always resulted in ANA in tumor serum through 11 successive transplants. A survey of other tumor sera has now provided evidence that ANA is not unique to BK tumors; it is present in a number of SV40 mouse and hamster sera, and in some polyoma tumor sera.

A complement-fixation test has been developed for further studies on the antigen and antibody. The antigen appears to be a heat-labile nucleoprotein. Absorption studies have shown that it is probably different from T-antigen; ANA activity in tumor serum can be adsorbed with soluble normal cell extracts without loss of T-antigen reactivity.

JCV replication: Despite numerous attempts to adapt JCV to growth in a variety of human cells, this virus still replicates only in human fetal brain cells (HFB). In HFB cells, the growth cycle is exceedingly slow and does not generally result in complete cell destruction. Surviving cells can be recovered and possess some of the properties of transformed cells, i.e., they have altered cell morphology and are 100% T-antigen positive; however, they do not have the capacity for infinite growth.

Significance to Biomedical Research and the Program of the Institute: The human papovaviruses constitute a group of viruses whose biology and significance in disease is poorly understood and requires investigation. One of them, JCV, has now been found to be most frequently associated with progressive multifocal leukoencephalopathy. Nothing is known about the association of BKV with human disease. Both of these viruses are oncogenic for animals and can transform cells in culture; they are, therefore, potential human oncogenic viruses.

Proposed Course of the Project: Biological and biochemical characterization of the human papovaviruses will be continued.

Publications:

Khoury, G., Howley, P.M., Garon, C., Mullarkey, M.F., Takemoto, K.K. and Martin, M.A. Homology and relationship Between the genomes of papovavirus BKV and SV40. Proc. Nat. Acad. Sci., 72:2563-2567, 1975.

Howley, P.M., Khoury, G., Byrne, J.C., Takemoto, K.K. and Martin, M.A. Physical map of the BK virus genome. J. Virology, 16:959-973, 1975.

Takemoto, K.K. and Martin, M.A. Transformation of hamster kidney cells by BK papovavirus DNA. J. Virology 17:247-253, 1975.

Mason, D.H. and Takemoto, K.K. Complementation between BK human papovavirus and an SV40 tsA mutant. J. Virology 17:1060-1062, 1976.

Takemoto, K.K. Human papovaviruses: Current knowledge on their biologic and oncogenic properties. Int. Symp. on Comp. Leukemia and Related Diseases. S. Karger (In press).

Howley, P.M., Khoury, G., Takemoto, K.K., and Martin, M.A. Polynucleotide sequences common to the genomes of simian virus 40 and the human papovaviruses JC and BK. Virology (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 A1 00019-02 LVD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Treatment of Disease with the Interferon System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dr. Hilton B. Levy, LVD, NIAID
Mr. Charles Buckler, LVD, NIAID
Mr. Freddie Riley, LVD, NIAID
Dr. Eduard Lvovsky, LVD, NIAID (Visiting fellow)

COOPERATING UNITS (if any) Dr. George Baer, CDC; Maj. Edward Stephen, Capt. Lin Sammon, Capt. Ed Houston, Ft. Detrick; Dr. F. Danzani, U. of Turin Med. School Dept. of Medicine; Dr. M. Lerner, Wayne St. U. Med. School of Medicine; Dr. H. Kaufman, U of Florida; Dr. Bektimeroff, Moscow; Dr. Purcell, Dr. Dolin, NIAID, Dr. Levine, NCI.

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Virology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

SUMMARY OF WORK (200 words or less - underline keywords)

An interferon inducer, based on the stabilization of poly I-poly C against nucleolytic destruction, has been produced. It has been shown to be a good interferon inducer, and an effective antiviral agent in non human primates against a number of serious virus diseases prophylactically and therapeutically. In man, it induces significant levels of interferon. In open trials in man strongly suggestive evidence of antiviral action has been seen. Double blind trials are underway.

Project Description:

The aspect of the section's program dealing with therapy of disease has proceeded in several directions. In a collaborative program, this section, primarily Mr. Buckler, Dr. Anfinsen's laboratory, and the National Cancer Institute at Fort Detrick are attempting to produce very large quantities of interferon. This interferon will serve two purposes. a) As a starting material for Dr. Anfinsen's interferon purification program, and b) as a possible therapeutic agent to be used in cancer patients at the National Cancer Institute. This program is currently occupying itself with establishing the best methods for the production of interferon in lymphoblastoid cell lines.

A major effort has been directed to initiating conditions for the trial in man of the interferon inducer we have developed. In monkeys, the drug (polyinosinc acid.poly cytidylic acid.poly-L-lysine.carboxymethyl cellulose = poly ICLC) has proven highly effective both prophylactically and therapeutically against Simian hemorrhagic fever, street rabies, yellow fever, vaccinia and Russian spring summer encephalitis viruses. In the last mentioned disease, studied in collaboration with Russian investigators, the virus was administered intracerebrally and complete protection was obtained. This indicates that the drug or its effect apparently is able to get past the blood brain barrier. The drug has been curative against herpetic keratoconjunctivitis in owl monkeys. In chimpanzees with chronic active hepatitis the disease is suppressed during treatment, but so far the disease has reappeared when treatment was stopped.

Two other observations have been made that are being actively pursued: 1) An ointment containing poly ICLC has been prepared. It has proven highly effective in controlling cutaneous vaccinia infections in rabbits both prophylatically and even when treatment began after the lesions were visible. Accompanying this action is the production of interferon in the skin. There is also present modest levels of interferon in the serum of treated rabbits. Uninfected monkeys treated with the ointment also show low levels of serum interferon. 2) Poly ICLC has shown strong immunoadjuvant properties in mice, rats and monkeys. Control monkeys receiving killed Venezuelan equine encephalitis vaccine developed antibody titers of 80 with levels of antibody dropping below detectability after about 25 days. When the vaccine was given along with poly ICLC, antibody titers reached 18,000 and were still close to that figure after 72 days.

Several trials in man have begun. Dose response studies have indicated that at levels of 0.2 to 0.3 milligrams per kilogram of body weight, peak levels of up to 800 units per milliliter of serum are seen. There have been dramatic recoveries in several

open studies of Herpes Type 1 and 2. A double blind study is underway using the ointment against Herpes Genitalis. A chronic toxicity study in monkeys, consisting of intravenous injections three times weekly, has been going on for three months. No untoward effects have been noted.

To date, no success has been obtained in inducing interferon in nasal secretions of man with topical application of the drug. Interferon has been produced in human tears after installation of drops in the eye of volunteers.

Future plans include expansion of the current human trials and further exploration of the immune potentiating capacity of the drug in primates and in man. Initiation of an additional study against tumors in man and against herpetic keratoconjunctivitis in man is also planned.

A new class of interferon inducer has been found in some of radioprotective agents known as aminothiols (Dr. Lvovsky). These compounds are effective orally in mice, both as interferon inducers and as antiviral agents, and in preliminary experiments appear to induce low levels of interferon in monkeys when given orally. This work will continue.

Publications:

W.B. Levy, C. Baer, S. Baron, C.F. Buchler, C.J. Gibbs, M.J. Iadarola, W.T. London, and J. Rice. A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. J. of Infectious Diseases, Vol. 132, No. 4, 1975.

W.B. Levy, W.T. London, D.A. Puccillo, S. Baron, and J. Rice. Prophylactic protection against simian hemorrhagic fever virus in monkeys by an interferon inducer, Poly I.Poly C-Poly-L-lysine. J. of Inf. Dis., June 1976, In press.

W.E. Houston, C.L. Crabbs, E.L. Stephen, W.B. Levy. Modified polyriboinosinic-polyribocytidylic acid--an immunologic adjuvant. Infection and Immunity, 1976, In press.

R.A. Robinson, V.T. DeVita, W.B. Levy, S. Baron, S.P. Hubbard, A.S. Levine. A phase I-II trial of multiple-dose polyriboinosinic-polyribocytidylic acid (poly I.PolyC) in patients with leukemia or solid tumors. J. National Cancer Institute, 1976, In press.

F. Dianzani, W.B. Levy, S. Berg, S. Baron. Kinetics of the rapid action of interferon. Proceedings of the Society for Experimental Biology and Medicine, 1976, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00020-09 LVD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Study of the Factors, in vivo and in vitro which Modify the Outcome of Chroni Virus Infections, Using Lactic Dehydrogenase Virus (LDV) and its Host Cell: The Mouse Macrophage as a Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Virology Section

INSTITUTE AND LOCATION
NIH, NIAID, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

Project Terminated

PHS-NIH
Summary Statement
Laboratory of Streptococcal Diseases
July 1, 1975 through June 30, 1976

Research Progress

Endocarditis Sera and Staphylococcal Teichoic Acids

As previously reported, sera from patients with staphylococcal endocarditis react with both teichoic acid (TA) and lipoteichoic acid (LTA) from Staphylococcus aureus. The TA is a polymer of ribitol phosphate, whereas LTA is a polymer of glycerol phosphate linked to lipids and alanine: either may also include sugar residues as side chains. The basis for the cross-reactivity is unclear, and one approach to the problem is to utilize highly purified preparations of TA and LTA as antigens to eliminate the possibility of co-concentration of cross-contaminants in prior tests. Such purified preparations were made, using purified mesosomal vesicles as LTA source and highly purified cell walls (free of membrane components) as source of TA: they are in final stages of testing for glycerol and ribitol prior to serologic tests with human sera. Concurrently, it was found that rabbits immunized with mesosomal vesicles produced antibody reactive in immunodiffusion tests with LTA, but not with TA; and the reaction was inhibited by LTA. In another system (passive hemagglutination to LTA-coated RBC), the serum reactivity was inhibited by LTA, but not by TA nor by triglycerol diphosphate. The latter rules out the polyglycerol phosphate backbone of LTA as antigenic determinant of the hemagglutination. Because periodate treatment of LTA destroyed the serum-inhibitory capacity of the LTA, a carbohydrate determinant is probably involved. This determinant is clearly not in common with TA and, by analogy, the results eliminate one possible explanation for the reactivity of human encodarditis sera with both TA and LTA. (Huff, Theodore, Beining)

Bacterial Lipoteichoic Acid

Prior work established the mesosomes (intracellular membranous organelles) as the sole site of lipoteichoic acid (LTA) in Staphylococcus aureus--in contrast to reports of periplasm and cytoplasmic membrane localization, as well as traverse of the cell wall and excretion of large amounts into the medium, in some streptococci and lactobacilli. Reinvestigation, by several sensitive chemical and serological methods, has verified the absence of LTA in S. aureus culture fluids at all stages of growth except late stationary log phase, in which only trace amounts (0.2% of total cellular LTA) were detected. In preparation for final proof of the intracellular location, by immunoelectron microscopy on sections cut from water-soluble embedments, antisera to purified LTA were prepared, tested for activity and specificity, and are being ferritin-conjugated by improved techniques. The importance of LTA, which has a basic polyglycerol phosphate structure in its common occurrence in gram-positive bacteria; its probable precursor relationship in synthesis of teichoic acid (TA) (an important lipid-less polyribitol phosphate component of the cell wall); and relative ignorance of, and controversy concerning, the real functions of either component. (Theodore, Huff, Beining)

It has been suggested that mesosomes including their vesicles which can be isolated are artefacts resulting from a time-dependent phase transition of intracellular components induced by fixation or by other manipulations or conditions. Such latter might include final growth level (culture age); media employed; conditions of protoplasting (for release of mesosomal vesicles), including concentration of lytic enzyme and total time of the procedure; and time and/or g-force of centrifugation for separation of vesicles. Differences in these conditions or procedures (old and young cultures; different medium concentrations of glucose or glycerol; increased concentration of lytic enzyme; centrifugation at 59,000 x g for 10 min, instead of 6,600 x g for 30 min) did not alter the yield of mesosomal vesicles. The artefactual production of these structures therefore remains in some question. (Huff, Theodore)

Phage and Lysogeny in Group A Streptococci

Only 2 of 102 strains of Group A streptococci exposed to mitomycin C failed to produce phage particles detectable by electron microscopy. Both non-lysogenic strains are of M antigen-type 23, though from different geographic sources and isolated in different years: 2 other M-23 strains contained prophage inducible by mitomycin C. Both non-inducible strains were successfully lysogenized by a phage from another streptococcal strain and then compared, by a variety of characteristics, with the parent strains, to observe any changes possibly due to lysogenic conversion. No alterations were detected in colonial morphology, hemolytic pattern, presence of capsule, nor in group- and type-specific serological reactions. However, one of the non-lysogenic strains appears to produce an erythrogenic toxin (as detected by skin tests, and verified by neutralization by specific antibody)-a situation presumed, on the basis of prior work of others, to be due to lysogenic conversion by the presence of a prophage. Either there are other and unsuspected means of genetic coding for toxin production, or a prophage (or some segment thereof)that is undetected by our repeated tests, is present in the host cell: confirmatory tests are in progress. In other approaches to the possible role of lysogenic conversion, several lysogenic strains of streptococci were induced by mitomycin C and ultraviolet irradiation in the presence of antisera specific for the homologous inducible phage: surviving colonies were picked, and the process repeated several times. "Curing" was based on subsequent absence of immunity to the homologous phage, and on failure of induction methods to produce phage detectable by either plaquing or indicator strains or by electron microscopy (EM). One strain fulfilled all criteria except absence of phage particles by EM, and it was concluded that naturally-occurring lysogenization with several phages--of which at least one is defective or is unable to replicate on the indicator strains tested--may account for the present problems of "curing." Because of these failures, it is as yet impossible to assess loss or alteration of streptococcal attributes that may have been determined by the presence of a prophage. Another possible test of effects of lysogeny was made by attempting DNA-mediated transformation of the non-lysogenic M-23 strains, because such transformation is known to be inhibited by lysogeny in some other bacteria: neither these, nor lysogenic strains (as repeatedly shown in the past) were transformable, and the reasons for failure of this genetic transfer mechanism in Group A streptococci remain unknown. (Colon, Popkin, Cole)

LABORATORY OF STREPTOCOCCAL DISEASES

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Genetic Competence and pH in Streptococci

DNA-mediated transformation in Group H strpetococci (Streptococcus sanguis) involves a series of steps that are, sequentially: (1) uptake of competence factors (CF) that are polypeptides; (2) induction of a cell-wall splitting autolysin; (3) achievement of a state of competence, which is (4) the capacity to bind, take up, and incorporate added DNA bearing a detectable genetic marker. Because autolysin has a pH optimum of 9.0 and is non-functional below pH 7.0, effects of lower pH on reaction of CF with cells, was tested. Under such conditions, no uptake of CF, and no autolysis (tested in the appropriate detection system), occurred. Since achievement of competence was previously shown dependent on autolysin, these results show the requirement of CF utilization for autolytic activity and subsequent competence. A second, possibly pH-sensitive step, is uptake of DNA. As tested by use of tritiated DNA added to competent cells, DNAase-sensitive binding was found maximal at pH 6.0, whereas DNAase-resistant (or irreversible) incorporation was optimal at pH 7.0. However, optimal transformation itself took place at pH 6.5. These findings suggest different pH optima for activities of perhaps several enzymes, as yet theoretical, that must be involved in the steps outlined. Future work will attempt to define such enzymes to further clarify the mechanisms for uptake and integration of high molecular weight DNA. (Ranhand)

Mycoplasma (Spiroplasma) Viruses

The distribution of 3 virus-like particles (VLP) previously described here by electron microscopy from Spiroplasma citri (a plant pathogen), was determined in 3 other microorganisms now recognized as spiroplasmas. The VLP are SVC1, a rod-shaped particle; SVC2, a long-tailed phage-like particle; and SVC3, a short-tailed phage-like particles. Of 4 cultured strains of the corn-stunt organism, two contained byth SVC1 and SVC3,. Of 2 cultured strains of the suckling mouse cataract agent (SMCA), each contained SVC3 only. One strain of the sex-ratio organism of Drosophila, examined in insect hemolymph because it has not yet been cultured, contained both SVC1 and SVC3. The SVC2 particle has, so far, been detected only in S. citri; and the presence of one or more VLP (sometimes all three) in 27 strains of S. citri was previously documented. The finding of VLP in SMCA, the most recently recognized and cultured spiroplasma and the first associated with mammalian disease, supplied additional evidence of spiroplasma relatedness. Concurrently, optimal methods were achieved for propagation of SVC3 from S. citri as plaques on lawns of S. citri, and were also used for SVC3-containing fluids form SMCA cultures. Present efforts are directed to production and purification of SVC3, in order to study biological and physicochemical characteristics of a virus-host system comprising a phage-like virus and a membrane-bounded wall-less prokaryotic organism. (Cole, Mitchell, Popkin; Tully, LID)

Administrative, Organizational, and Other Changes

The Laboratory of Streptococcal Diseases completed its third year of existence as a separate entity. For most of the year, the Laboratory Chief was virtually absent while acting as Scientific Director, Laboratory and Clinical Research, NIAID. The Laboratory continued to operate 7 projects by 4 other investigators and 3 GS-11's, with the aid of 6 technical personnel. Two others (Mr. Gary Best, WW1; and Mr. Robert Bradley, Biologist GS-7) left NIH during the year. Dr. Harvey L. Edmonds, Research Associate, completed his 2-year assignment on June 30, 1976. Dr. Gary Calandra finished his first year's residency in Internal Medicine, in training outside the service, at Parkland Memorial Hospital (University of Texas Health Center) at Dallas.

Decreased numbers and quality of applicants for the Associate Program, and particularly in those expressing interest in LSD program, resulted in failure of selection of a Research Associate for July 1, 1976. The same pattern is noted in applications, now being reviewed, for Associates to enter July 1977 or later. It is suggested that the restriction seemingly expressed by Laboratory name, as well as the small size of the Laboratory and consequent small number of projects, may be in part responsible for lack of interest among prospective candidates. A change of name, representative broader interests and potential, should be considered.

While the mission of the Laboratory of Streptococcal Diseases is considered to be based--historically and to some extent on past accomplishments and present functions--on interest in streptococci, it is a fact that some interests of present (and future) investigators do (and will) involve other microorganisms. Of 7 current projects, only 2 (transformation genetics and competence; bacteriophages and transduction genetics) involve streptococci. Two deal primarily with staphylococci; one with gonococci; one with mycoplasma (spiroplasmas) and their viruses; and one with many aspects, including support functions of microbial ultrastructure. Therefore, the mission should be more broadly construed as contributing to knowledge of structure and function--not only in streptococci, but also in other microorganisms in which cell walls, membranes, intracellular organelles, biologically active molecules, bacteriophages, pathogenesis, antigens, and genetic mechanisms, can be studied.

Honors and Awards

Roger M. Cole

Member, Editorial Board, Infection and Immunity.

Member, Editorial Board, Antimicrobial Agents and Chemotherapy.

Project Officer: NIAID Research Contract PH 43-68-83, University of Chicago.

Faculty, Mycoplasma and Cell Culture Workshop, W. Alton Jones Cell Science Center, Lake Placid, N. Y.; Dec. 8-10, 1975.

Jon M. Ranhand

Invited participant, XIX Annual Meeting on Microbial Transformation, Estes Park, Colorado; June, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00001-15 LSD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on Staphylococcal and Streptococcal Membranes, Cell Walls, and Mesosomal Vesicles.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	E. Huff	Scientist Director	LSD NIAID
Others :	T.S. Theodore	Research Microbiologist	LSD NIAID
	R.M. Cole	Chief, Lab. of Strept. Dis.	LSD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.00	0.75

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the biochemical processes involved in the growth of bacterial cell wall and membrane. Of particular interest is the biosynthesis of cell wall teichoic acids and mesosomal lipoteichoic acids in Staphylococcus aureus. The topics of present interest are 1) the theory that lipoteichoic acid occurs in the mesosome of Gram-positive bacteria; 2) the relationship of the high content of lipoteichoic acid in mesosomes to the synthesis of teichoic acid in the cell wall; 3) improvement in methods to isolate mesosomes; 4) the specificity of antibodies in endocarditis patients toward lipoteichoic acid and teichoic acid.

Project Description:

Teichoic acids are highly antigenic polyribitol phosphates located on the exterior surface of Staphylococcus aureus and many other Gram-positive cell walls. The purpose of this study has been to: 1) investigate the biochemical mechanisms by which these polyribitol phosphates are formed and attached to the cell wall structure; 2) to relate the occurrence and structure of teichoic acids to the strong immunological response observed during staphylococcal infection.

We have reported previously that lipoteichoic acid (LTA), a glycerol phosphate polymer reported by others to occur in bacterial membranes, was localized in the mesosomes (intracellular membranous organelles comprising 12% of the total intracellular membrane) of S. aureus. Because of the importance of LTA as a bacterial antigen and as an assembly point for the synthesis of cell wall teichoic acid (TA), it is important to establish whether its localization in mesosomes is a general bacterial phenomenon or a pecularity of S. aureus. As reported previously, of 7 Gram-positive bacteria tested in all cases (except M. lysodeikticus, where a lipomannan is present instead of lipoteichoic acid) the mesosomes have 1) a threefold or greater concentration of LTA than in the membranes; 2) the major portion of the total cellular LTA. To establish more completely the general nature of the localization of LTA in mesosomes of Gram-positive bacteria we are examining several streptococcal strains: S. faecalis (where a controversy exists regarding the localization of LTA); S. mutans BHT (an excessive producer of extracellular LTA, and S. sanguis. In addition we plan to test Staphylococcus aureus H, and Lactobacillus fermenti (two strains that have been used for studies of lipoteichoic acid).

It has been suggested by others that mesosomes may be formed by a time dependent phase transition of membranes during fixation for electron microscopy. Thus, one might expect that variations in the 1) final growth level; 2) growth media; 3) protoplasting time; 4) centrifugation time of the protoplasts, might affect the yield of mesosomes. The yield of mesosomes from S. aureus (Lafferty and 6538P) is unaffected when a) the protoplasting time is reduced from 1 hour to 10 minutes by using an elevated lysostaphin concentration; b) the protoplasts were removed by a 10 minute centrifugation (59,000 x g) instead of a 30 minute (6,600 x g) centrifugation. In synthetic media the mesosome yield was not affected by the final growth level or the presence of various concentrations of added glucose or glycerol. The constancy of mesosomal yield under a variety of cultural conditions and protoplasting procedures puts serious constraints on the possibility that mesosomes are protoplasting artifacts.

Fiedler and Glaser show that LTA in S. aureus H acts as a template for the synthesis of a polymer (LTA-TA) in which LTA is attached to TA. This molecule is presumably an intermediate in the synthesis of cell wall teichoic acid. In their enzyme preparations from "membranes" these authors did not distinguish between mesosomes and membranes. We wish to test for the synthesis of LTA-TA by both mesosomes and membranes in order to determine if mesosomes, as

indicated by their high levels of LTA, are of primary importance in the synthesis of cell wall teichoic acids. In this regard we are currently involved in the chemical synthesis of cytidine diphosphate (C14) ribitol, the precursor for teichoic acid synthesis.

As discussed in our 1974-1975 annual report, sera from staphylococcal endocarditis patients reacts not only with teichoic acid but also with lipoteichoic acid. We are attempting to determine whether these results are due to specific or cross-reacting antibodies or to cross contamination of LTA and TA preparations. In order to eliminate the problem of cross contamination we have purified LTA from mesosomes of S. aureus (Lafferty and 6538P) by Sepharose chromatography. Teichoic acid was purified from S. aureus (Lafferty, 6538P, and H) by lysis of cell walls (treated in various ways to remove any membranous material) with lysostaphin followed by chromatography on Sepharose. These preparations will be tested for ribitol and glycerol content and cross reactivity with the LTA preparations against endocarditis sera. Serum from rabbits immunized with S. aureus mesosomes reacts with LTA but not TA by immunodiffusion. The reaction of this antisera with LTA is inhibited by LTA but not by triglycerol diphosphate or crude TA (S. aureus, Lafferty, cell wall lysostaphin lysate) as tested in the passive hemagglutination reaction. Thus, in rabbit antisera, the antibody specificity is not directed to the polyglycerol phosphate backbone of LTA nor does it react with cell wall TA. The specificity site of LTA (as an inhibitor in the passive hemagglutination reaction) is probably a carbohydrate because it is destroyed by periodate.

The teichoic acids are major structural components of the Gram-positive bacterial cell wall and strong antigens in the human. Lipoteichoic acids are antigenic molecules involved in the biosynthesis of teichoic acids. At present we are studying 1) the theory that lipoteichoic acid occurs in the mesosome of most Gram-positive bacteria; 2) the relationship of the high content of lipoteichoic acid in mesosomes ot the synthesis of teichoic acid in the cell wall; 3) the specificity of antibodies in endocarditis patients toward lipoteichoic acid and teichoic acid.

Publications:

Cole, R. M., Caladnra, G. B., Huff, E., and Nugent, K. M.: Attributes of potential utility in differentiating among "Group H" streptococci or Streptococcus sanguis. J. Dent. Res. 55: A142-A153, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00002-11 LSD

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the nutrition, physiology and ultrastructure of Staphylococcus aureus.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. S. Theodore	Research Microbiologist	LSD NIAID
Others:	E. Huff	Scientist Director	LSD NIAID
	R. M. Cole	Chief, Lab. of Strep. Dis.	LSD NIAID
	P. Beining	Guest Worker	U. Scranton

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	1.3	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The overall purpose of this project is to correlate membrane structure with function in Gram positive bacteria using Staphylococcus aureus as the model test system. Of particular interest is to define the function (or functions) of mesosomes. The topics of present interest are: chemical and immunochemical localization of lipoteichoic acid in S. aureus; characterization of the structure of staphylococcal lipoteichoic acid; and analysis of the steps involved in lipoteichoic acid biosynthesis and its possible function as related to the mesosome.

Project Description:

The membrane system of S. aureus and other Gram positive bacteria consists of a plasma membrane and mesosomes (intracytoplasmic membranous organelles). The objective of this study is to isolate the two membrane fractions and analyze their ultrastructure, chemical composition and enzymatic activities in an attempt to determine the function (or functions) of mesosomes.

Staphylococcal mesosomes appear unique in that they contain all the lipoteichoic acid (LTA) of the cell; however, in other Gram positive bacteria this polymer has been also associated with the plasma membrane, periplasm and/or cell surface (detectable in culture fluids). By chemical analysis, we have shown that this polyglycerol phosphate polymer is associated in S. aureus, almost exculsively with mesosomes and only trace amounts are detectable in the plasma membrane, cytoplasm and periplasm. Also, studies on various parameters of protoplast formation, mesosome isolation and LTA extraction show that LTA is still present in mesosomes. Analysis of culture fluids obtained from different stages of the growth cycle showed that no LTA is present in early, mid and late log phase culture fluids, and only trace amounts are detectable in late staionary phase culture fluids.

Attempts to prepare antisera for the immunoelectronmicroscopic localization of LTA in whole cells were partially successful. Staphylococcal cells were disrupted by Braun disintegration, the LTA extracted with chloroform-methanol (2/1), and hot water (80°), and purified by column chromatography on sepharose 6b. The LTA obtained as one single peak was judged homogeneous by; glycerol:phosphate ratio of 1, absence of 260nm absorbing material, a single protein band on polyacrylamide gels which stained positively with Schiff-periodic acid reagent, and the presence of a single precipitin band when reacted with mesosomal antisera. This material is highly immunogenic, in contrast to preparations extracted with aqueous phenol (40%): immunogenicity appears due to the high protein content (45%) of hot water extracts as compared to low protein (15%) in aqueous phenol extracts. Antisera produced by immunization with hot water extracts, when tested against phenol-extracted purified LTA showed only one precipitin band and failed to react with cytoplasm or periplasm. Concentrated culture fluids from early, mid and late log phase cells also failed to react with the antisera, and late stationary phase culture fluids reacted only feebly. For ferritin conjugation studies, the IgG fraction of the LTA antisera was isolated by $(NH_4)_2SO_4$ fractionation and DEAE cellulose chromatography. The IgG fraction was eluted as a single peak, concentrated with aquacide, and conjugated to ferritin with either fluoronitrophenyl sulfone (one stage conjugation) or tolylene diisocyanate (two stage conjugation). Although the ferritin-labelled IgG fraction (one-stage) gave a single strong precipitin band with purified LTA, its binding to thin sections of methacrylate-embedded whole staphylococci appeared non-specific and no distinct zones of ferritin, indicating localization of LTA, could be seen in any portion of the cell.

Chemical analysis under a variety of conditions as well as the partial serological characterization of all of the isolated subcellular fractions of S. aureus demonstrate that LTA is associated with the mesosomes. Although the data on the serological localization of LTA in situ is still lacking, we have succeeded in preparing antisera to LTA. Continued efforts on the immunochemical localization of LTA should finally resolve the problem of whether LTA is associated with mesosomes and permit us to extend these investigations as to the possible involvement of mesosomes in membrane and cell wall biosynthesis.

The planned course of this project will include continued efforts on the immunoelectronmicroscopic localization of LTA in intact cells of S. aureus by use of affinity column chromatography techniques for the purification of anti-LTA-IgG, and of improved methods of ferritin conjugation. The steps involved in LTA biosynthesis, and its function as it relates to the mesosomes, will also be studied.

Publications:

Nugent, K.M., Calandra, G.B., and Theodore, T.S.: Cellular location of alpha-hemolysin in Staphylococcus aureus. Inf. & Immun. 12: 270-273, 1975.

Calandra, G.B., and Theodore, T.S.: Cellular location of streptolysin O. Inf. & Immun. 12: 750-753, 1975.

Chirigos, M.A., and Theodore, T.S.: The effect of immune modulators on the oncongenic process: adjuvant effect of Staphylococcus aureus in treating murine luekemia. Ann. N.Y. Acad. Science. International conference on immunology of cancer (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00003-3-LSD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characteristics of "Group H" Streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. B. Calandra	Medical Officer	LSD, NIAID
Other:	R. M. Cole	Chief of Laboratory	LSD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Because of reassignments (Dr. Calandra to training outside the service: Dr. Cole to Acting Scientific Director, NIAID), no work was performed on this project and it is terminated.

Publications:

Cole, R. M., Calandra, G. B., Huff, E., and Nugent, K. M.: Attributes of potential utility in differentiating among "Group H" streptococci or Streptococcus sanguis. J. Dent. Res. (Special Issue A) 55: A142-A153, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00004-16 LSD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Electron Microscopy of Bacteria in Relating Structure and Function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Cole	Chief of Laboratory	LSD, NIAID
Other:	T. J. Popkin	Chemist	LSD, NIAID

COOPERATING UNITS (if any)

See following page for cooperating units.

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.2	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project provides support, through preparative techniques (negative staining, thin sectioning) and subsequent examination, for Laboratory and Collaborative microbiological studies requiring transmission electron microscopy. Objects of ultrastructural study include mycoplasmas and their viruses; streptococci and their bacteriophages; staphylococci; bacilli; gram-negative bacteria; fungi; and various cell fractions thereof, examined by means of cytochemistry and immunoelectron microscopy.

Cooperating Units

University of Arizona, College of Liberal Arts, Dept. of Microbiology and Medical Technology, Tucson (Dr. N. H. Mendelson)

Tufts University School of Medicine, Dept. of Microbiology, Boston (Dr. J. T. Park)

Bureau of Biologics, FDA, Div. Bacterial Products (Dr. M. Barile, Dr. S. Rottem)

Lab. of Infectious Diseases, NIAID, Mycoplasma Section (Dr. J. G. Tully)

Lab. of Clinical Investigations, Clinical Mycology Section (Dr. K. J. Kwon-Chung)

Project Description:

Objectives are to provide, as required, electron microscopy facilities in support of other projects in the Laboratory; and to conduct such original and in depth studies of the ultrastructure of micro-organisms as may be engendered by collaborative beginnings or by independent approach to problems.

Because of the temporary assignment of the Principal Investigator, for 10 months of the year, as Acting Scientific Director, NIAID, project activity was considerably curtailed. Those studies carried out were related to collaborative and supportive efforts, as follows:

1. Continued monitoring of Spiroplasma citri and other spiroplasmas, involving several hundred specimens, was carried out as a essential part of a study on viruses of these mycoplasmas (See Project No. Z01 AI 00007-1 LSD). Examinations included both negatively-stained and sectioned materials.

2. Frequent examinations of mitomycin C-induced cultures of Group A streptococci were made, as an extension of prior work on the prevalence of lysogeny. Nearly 99% of strains are lysogenic: two apparently non-lysogenic strains (both of M-type 23, though from different sources) were found and are proving useful in other studies of effects of lysogeny and of methods of genetic exchange in streptococci. (See Project No. Z01 AI 00005-10 LSD)

3. Two strains of Group A streptococci, characterized as to M protein antigen production and localization (i.e., primarily cell-associated, or largely extra cellular) were subjected to different schedules and sequences of fixation and washing, to determine optimal methods for preservation and EM demonstration of the M-protein surface fimbriae in ultrathin sections. Best results were obtained by an initial phosphate-buffered saline wash, followed by fixation in glutaraldehyde-cacodylate, wash in cacodylate buffer, and osmium fixation in a medium without tryptone.

4. In attempts to demonstrate surface, cell wall, membrane/mesosome, or intracytoplasmic localization of antigens, two methods of labelling with ferritin-labelled antibodies were tested on Group H streptococci and staphylococci.

A. External application of labelled antibodies (either direct or by the "sandwich" technique) to whole cells, followed by washing, fixation, and embedment and sectioning in conventional epoxy resin (Spurr's medium), or

B. fixation of whole cells, followed by embedment in UV-polymerized water-soluble glycol methacrylate, sectioning, and exposure of cut sections to labelled antibodies.

Although good sections were obtained by both techniques, labelling was random and non-specific. The preparation of the labelled antisera is suspect, although several crude IgG fractions and different methods of conjugating several preparations of ferritin were used. Ultrapure ferritin and column-purified IgG are currently being tested. (In support of Project No. Z01 AI 00002-10 LSD)

5. Preparations of Bacillus subtilis, wild-type and mutant, were embedded, sectioned, and examined to determine the utility as a genome marker, of sequential treatments with sodium dodecyl sulfate, pronase, RNAase; and DNAase--in an extension of last year's collaboration with Dr. Mendelson (U. of Arizona).

6. Brief examinations were made of thin-sectioned Escherichia coli, involving a temperature-sensitive mutant that, at restrictive temperature (42 C) produces long filaments deficient (by chemical tests) in the lipoprotein that links peptidoglycan to outer membrane. Ultrastructurally, no differences in cell envelope structures were seen between cells grown at 30 C and those grown at 42 C. (Collaboration: Dr. Park, Tufts University School of Medicine)

7. As a basis for anticipated future studies, sections of Neisseria gonorrheae from both culture and fresh urethral exudates, were examined to obtain a series of morphological and ultrastructural controls. (See Project No. Z01 AI 00008-1 LSD)

8. A strain of human kidney cells (BSC-1) reputed in literature to be infected intracellularly by a mycoplasma, was supplied by Dr. Barile (BoB/FDA) in late phase culture. Thin sections showed no mycoplasma either intracellularly nor extracellularly, although cultural methods in BoB isolated 2 mycoplasmas--neither of which was the species originally purported to be present. A similar cell culture, to which a known mycoplasma (M. hyorhinis) had been added, revealed only extracellular and intercellular mycoplasma. The study is being extended to include cell cultures at increasing times of incubation--both as controls and after addition of several known mycoplasmas--to ascertain whether mycoplasmas can recognizably invade cells or replicate intracellularly at any stage of cell culture growth.

9. Negatively-stained preparations of several mycoplasma or their membranes treated with tetanolysin were examined in conjunction with Dr. S. Rottem (Visiting Scientist, BoB/FDA). Small holes, reminiscent of antibody-complement-induced lesions in red-cell or gram negative bacterial membranes, were seen occasionally; but never occurred in membranes treated with inactivated (heated) lysin. In an incidental finding, cells of 2 goat strains of mycoplasma

(now recognized as M. capricolum) were found to possess striated intracellular bodies previously described in some other caprine, and a few bovine, strains related to M. mycoides. These bodies, with a periodicity of 12-14.5 nm, are known to consist of 2 proteins, but their functional significance is unknown.

10. Examination of thin sections of Cryptococcus neoformans confirmed the presence of clamp connections with dolipores, of basidiomycete type, in the septa. Some examples of diploid nuclei were seen but sampling was inadequate. The data nevertheless supply additional evidence for the removal of C. neoformans from the Fungi Imperfecti, the existence of mating types, and its placement, in a new genus Filiobasidiella (as previously reported by our collaborator, Dr. J. Kwon-Chung, LCI).

Projects will be continued or curtailed, as appropriate to the needs.

Publications:

Mendelson, N. H., Keener, S., and Cole, R. M.: Growth of minicell-rod$^-$ double mutants of Bacillus subtilis. Microbios 13: 175-183, 1975.

Kwon-Chung, K. J., and Popkin, T. J.: Ultrastructure of septal complex in Filobasidiella neoformans (Cryptococcus neoformans). J. Bacteriol. 126: 524-528, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00005-11 LSD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Bacteriophages and Genetics of Streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alba E. Colón	Microbiologist	LSD, NIAID
Other:	R. M. Cole	Chief of Laboratory	LSD, NIAID
	T. J. Popkin	Chemist	LSD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	0.1	2.4

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study lysogenic(especially lysogenic conversion) means of genetic exchange (transduction, transformation) in Group A streptococci.

A high percentage (99%) of lysogeny was found. We are now investigating the characteristics, and possible changes therein, of streptococcal cells before and after the acquisition of a prophage. The characteristics studied are: (1) serological variability, (2) colonial morphology, (3) production of hemolysins and erythrogenic toxin, (4) ability to transform, and (5) ability to become a transducing donor or recipient.

Project Description:

The objective is to determine the role of lysogeny, or of lysogenic conversion, and of genetic exchange mechanisms (transduction, transformation) in streptococci and to try to elucidate how such mechanisms may affect important attributes such as antigenic characteristics,virulence and pathogenic factors.

Previously, 102 strains of Group A streptococci, representing 47 different serological types and non-typables, were tested by direct electron-microscopy (EM) examination of mitomycin C-induced preparations. All were found lysogenic except for 2 of 4 M-type 23 strains.Six strains were chosen for further studies. The phages of each were isolated, propagated to high titers and specific antisera were prepared against 3 of the isolated phages.

The two M-type 23 non-lysogenic strains, 64 x 402 and 52 x 30, are very similar in phage sensitivity and ability to be transducing recipients, but they differ in colonial morphology, 64 x 402 is a mucoid and 52 x 30 a matt colony.

In studying the effect of lysogeny in Group A streptococci, we sought to compare the characteristics and possible changes occurring in a strain before and after the acquisition of a prophage. Two methods were used to obtain the desired strain. One was to "cure" the lysogenic strain of its prophage, the other was to lysogenize the non-lysogenic strains. To "cure", 3 strains were repeatedly induced with mitomycin C and ultraviolet irradiation in the presence of specific phage antisera, and survivors selected. Cultures were considered "cured" when (1) the cells were no longer immune to the homologous phage and (2) upon induction of the culture, no bacteriophage was found when tested by either plaquing on the appropriate indicator strain or by direct EM examination.

As determined by all criteria, we were unable to "cure" any of the three strains. However, one strain (56 x 188) fulfilled requirements, but bacteriophages were still found by direct EM examination. It thus appears that the strain carries multiple prophages and this may be the cause of the difficulty encountered in "curing" them.

Six bacteriophages isolated from strains that have desirable characteristics (i.e., erythrogenic toxin production, transducing donor, lack of hemolysins and antigenic variations) were used to lysogenize the two non-lysogenic strains of M-type 23. All phages were readily adsorbed to the bacterial cells and two propagated to high titers in the non-lysogenic strains. The procedures used for lysogenization were: (1) high multiplicity of phages were added to early log, log, and stationary phase cells. After 3 hours incubation at 29 C and 37 C, the lysates were plated for survivors, (2) in order to obtain lysogenic mutants, log cultures were treated with nitrosoquanidine prior to the addition of phage. In both procedures, hundreds of colonies were tested for immunity to the homologous phage and for the release, upon induction, of the added phage. Of the six phages used, only one AT203U, derived from Group A strain C203U (M-type 3), established lysogeny in both M-type 23 strains.

The two non-lysogenic M-type 23 strains 64 x 402 and 52 x 30, and their lysogenic derivatives 64 x 402/AT203U and 52 x 30/AT203U, were compared for differences in their characteristics. No changes were found in colonial morphology, hemolysis production, presence of capsule, nor group and M-type antigens as detected by serology.

At present, we are studying production of erythrogenic toxin by the lysogenic and non-lysogenic strains. Erythrogenic toxin production by Group A streptococcal cells has been linked to the presence of a prophage (lysogenic conversion). Preliminary experiments suggest, unexpectedly, production of the toxin by non-lysogenic strain 64 x 402. Work is now in progress to repeat these results and, if confirmed, to explore other possible mechanisms of coding for toxin production.

In some bacteria (Bacillus subtilis, Streptococcus sanguis), genetic transformation is inhibited by lysogenization. Attempts were made to transform the two non-lysogenic strains, using Group A streptococcal DNA carrying the streptomycin marker. Competence factor derived from Group H strain Challis was added in some experiments. There was no transformation of the tested strains, indicating that still-undefined factors are responsible for the often-observed inability to transform streptococci of Group A.

These basic studies are supplying data designed to determine and clarify, in human pathogenic Group A streptococci, the role of altered or new genetic information--whether it be supplied by lysogeny, DNA-mediated transformation, or phage-mediated transduction. Such information can conceivably alter factors (M protein, toxins, hemolysins) affecting direct pathogenicity, unknown factors influencing such post-streptococcal sequelae as rheumatic fever and nephritis, or antibiotic resistance patterns. The genetics of Group A streptococci is relatively unexplored, and the nearly universal occurrence of lysogeny with phages that we previously showed capable of transduction, emphasizes the need for continued investigation in the directions indicated.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00006-5 LSD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Competence Development and Genetic Exchange Mechanisms in Streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jon M. Ranhand Sr. Scientist LSD, NIAID
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to study genetic transfer mechanisms (primarily transformation) in the Group H streptococci. This includes studying the physiology of competence development, the interaction of competent cells with deoxyribonucleic acid (DNA), recombination of DNA molecules and their subsequent expression. This study may also involve related bacteriophages and their effect on the above.

Project Description:

During the past year I examined the effect of pH (values ranging from 5.5 to 9.0) on (i) competence development and (ii) DNA uptake by competent cells. Earlier studies showed that competence in Streptococcus sanguis (Wicky) cells is associated with the action of a specific autolysin that is induced by undefined extracellular polypeptides known collectively as competence factors (CF). (These factors are found in culture fluids derived from another Group H strain known as S. sanguis [Challis].) The initial objective of this study was to further correlate the function of the CF induced autolysin with the competent state. Since the autolysin has a pH optimum around 9, and does not function below pH 7.0, competence should be prevented from developing at the low pH values if the autolysin plays a role.

When cells were treated with CF below pH 7.0 they developed little or no competence. When tested under permissive conditions, these cells did not autolyze. Further investigation revealed that at low pH values the cells did not take up CF. This result indicates that competence development and autolysis are related to the utilization of CF.

DNA is routinely added to competent cells in media at pH values around 7.4 to 7.6. When tritiated DNA was added to competent cells at various pH values (5.5 to 9.0) different optima were found for (i) binding (sensitive to DNAase) and (ii) irreversible incorporation (resistant to DNAase). The pH optimum for irreversible incorporation was 7.0; cells bound DNA maximally at pH 6.0; transformation occurred optimally at pH 6.5. These different pH optima may reflect activities of various enzymes, as yet undefined, that are involved in this series of complex reactions. Concomitant with irreversible DNA uptake, acid-soluble DNA fragments appeared in the menstruum. It is not yet known whether these fragments are associated with the actual uptake mechanism or result from recombination reactions. Their nature will be investigated.

These findings bring us closer to a basic understanding of the competence mechanism and how a cell takes up such a large molecule as DNA.

Continuation of the study of competence and genetic transformation in S. sanguis cells will involve examining suspect enzymes and specific mutants with the hopes of defining a mechanism.

Publications:

Ranhand, J. M.: Effect of pH on competence development and deoxyribonucleic acid uptake in Streptococcus sanguis (Wicky). J. Bacteriol. 126: 205-212, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00007-02-LSD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Viruses of Spiroplasma citri and Other Mycoplasmas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Cole	Chief, Lab. Strep. Diseases	LSD	NIAID
Other:	W. O. Mitchell	Biologist	LSD	NIAID
	T. J. Popkin	Chemist	LSD	NIAID

COOPERATING UNITS (if any)

Mycoplasma Section (J. G. Tully), LID (Z01 AI 0027-8-LID)

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	0.1	1.9

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to characterize and to determine the prevalence of three virus-like particles (VLP) previously detected by electron microscopy in Spiroplasma citri and now found in three other Mollicutes (Mycoplasmas) now recognized as spiroplasmas (i.e., corn stunt organism (CSO); sex-ratio organism (SRO) of Drosophila; and suckling mouse cataract agent (SMCA). The VLP are (1) SVC1, a rod-shaped particle: (2) SVC2, a long-tailed phage-like particle; and (3) SVC3, a short-tailed particle. S. citri contains all 3 VLP; CSO and SRO contain 2 VLP each (SVC1 + SVC3); and SVC3 only was found in SMCA. Optimal methods were developed for plaque (infectious center) formation on agar lawns of S. citri by SVC3--from both S. citri and SMCA cultures as virus sources. Titers of 1×10^9 plaque-forming units (PFU)/ml. can be obtained. Purification methods, being investigated, will permit biological and physicochemical characterizations of this new virus-host system.

Project Description:

Objectives are to define the prevalence, morphologies, and biological, and physiochemical properties of virus-like particles (VLP) occurring in recently-discovered mycoplasmas--of which Spiroplasma citri, a plant pathogen, is the prototype genus and species. Three morphologic types of VLP were previously described from S. citri: SVC1, a rod-shaped particle; SVC2, a long-tailed phage-like particle; and SVC3, a short-tailed phage-like particle.

Methods have employed, almost exclusively, electron microscopy of negatively-stained host-cell culture preparations to detect and classify the virus-like particles by morphologies. During this year, in addition, we have developed a successful technique for growing host-cell (S. citri) lawns on agar, and for infecting these with a virus for the detection and enumeration of infectious centers (plaques) resulting from virus replication.

Major findings relate, in large part, to factors of importance to the plaquing technique. Of several media tested, one was selected as most consistent. Other factors include: (1) the age of the broth culture used for lawn production (2-3 days, log culture, optimal); (2) time of incubation of lawn at 32 C., prior to spotting virus-containing fluids (6 hr. optimal); (3) time of incubation before plaques become visible (best at 4-5 days); (4) age of broth culture from which optimal yields of virus can be obtained (usually older, or post-log cultures, at 5-7 days). Using a single strain of S. citri (608) as virus source, 10 strains of S. citri were tested for plaquing ability, and 5 were satisfactory. Other virus sources produced negative or inconsistent results on all lawns. One lawn strain (750) was most consistent (67% plaques in 500 tests from all virus sources; 94% plaques when the virus source was strain 608), and is now used routinely. Saline or water washes of these plaques reproduced plaques of new lawns: both washings and broth culture supernatants produced titers as high as 1×10^9 plaque-forming units (PFU) per ml. Electron microscopy of these revealed only one type of VLP, which was morphologically consistent with SVC3.

In broth medium at room temperature, SVC3 is viable (as determined by plaque formation) for at least 40 days: no quantitation of titers has yet been performed. Attempts to induce virus by treatment of broth cultures with mitomycin C, continue to be unsuccessful: appearance of virus is spontaneous, but other inducers are being tested. Trials to improve plaque visibility by several dyes were also unsuccessful, as were initial attempts to purify and concentrate SVC3 by detergent lysis of host cells, use of polyethylene glycol, and cesium chloride and sucrose density gradient centrifugations.

A new discovery was the finding of an SVC3-like VLP in the suckling mouse cataract agent (SMCA). This microorganism, originating from a rabbit tick, long passed blindly in eggs, and known to cause cataracts in suckling rodents, was recently recognized as a spiroplasma and cultivated in liquid artificial media by Dr. Tully and co-workers (Mycoplasma Section, LID). The virus is detectable in egg-passaged material, but is produced abundantly in broth cultures: only this, and none of the other two VLP found in other spiroplasmas, has been detected in the SMCA. It is therefore a better source

of a single virus, and efforts are now concentrating on its study. Preliminary results show that it will form plaques on lawns of S. citri: of 20 S. citri lawns tested, 3 (including strain 750) produced plaques. Agar lawns of SMCA have not been produced--but, when available, reciprocal testing of lawns and SVC3 from both S. citri and SMCA will be conducted.

The finding of common VLP is of significance, in addition to other attributes, in relating the four microorganisms now recognized as spiroplasmas. S. citri, the corn stunt organism (CSO), and the sex-ratio organism (SRO) of Drosphila, all contain 2 or more of the VLP, whereas the SMCA contains at least one. The occurrence of a newly-recognized group of mycoplasmas with pathogenic capabilities in plants, arthropods, embryonated eggs, and mammals, is of general interest in microbiology. Our future efforts will be directed to production of the VLP, in order to supply means for determining host-ranges, serological relatedness, and a new system utilizing a non-walled microorganism for investigating basic aspects of the biology of phage-like viruses.

Publications:

Cole, R. M.: Spiroplasma viruses. In Dalton, A. J., Hagenau, F., and Maramorosch, K. (Eds.): Insect and Plant Viruses: An Atlas. New York, Academic Press, Inc. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00008-2 LSD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Pathogenesis of Gonococcal Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. L. Edmonds	Research Associate	LSD, NIAID
Other:	R. M. Cole	Chief of Laboratory of Streptococcal Diseases	LSD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to study the role of human immunoglobulins in the pathogenesis of gonococcal urethritis, with emphasis on secretory immunity. The distribution of immunoglobulins in urethral pus is to be determined by indirect immune electron microscopy using ferritin-conjugated antibody as a tracer. For this purpose, a study has been make of the chemical properties of ferritin; immune globulins have been isolated by immunoabsorption; ferritin conjugation (with subsequent chromatographic isolation of pure conjugate) is being performed.

Project Description:

The purpose of the project is to examine host parasite relationships in gonococcal urethritis. By studying clinically acute urethral exudates we intend to: determine the distribution of antigonococcal immunoglobulins on gonococci within host cells as well as in the surrounding milieu; contrast the presence of 11s with 7s immunoglobulins; compare the extent of antibody binding to piliated, non-piliated, and cell wall deficient organisms; and finally to learn the extent to which immunoglobulin binding conincides with invasion of host cells and/or phagocytosis.

The technique chosen for these studies in indirect immune electron microscopy with ferritin conjugated antibody. Commercially prepared ferritin conjugated antibodies were found lacking in affinity and/or specificity. Thus, it was decided to purify and conjugate the immunoglobulins within the laboratory. A two stage conjugation procedure was chosen to widen its applicability to other projects and to reduce the level of background staining. Inadequacies of commercial conjugates were traced in part to five problems. First, the size of the immune globulin fraction of a given IgG pool is usually limited to less than 5%. Second, immune globulins themselves possess heterogeneous affinities for a given ligand. Third, when IgG of unwanted specificity is incorporated into conjugate, undesirable background staining is enhanced. Fourth, even when immune globulin is conjugated to ferritin, titer reductions result from incomplete conjugation and blockage of Fab sites within the conjugate. All of these factors limit antigen binding capacity as expressed per milligram IgG. Finally, failure to remove polymerized ferritin, free activated ferritin, and free immune globulin from the conjugation reaction mixture causes tracer aggregation, and discontinuous staining by competition for ligand by free IgG.

Ferritin

Commercially prepared ferritin preparations of the highest grade from several manufacturers, when analyzed chromatographically on 10% agarose were found to contain ferritin with varying iron saturation, unacceptable levels of apoferritin, free ferric salts in solution, or combinations of these. These impurities may not interfere with conjugation but do produce a conjugate that, by virtue of unpredicatable iron saturation, produces non-quantitative staining of variable intensity.

Immunoglobulin

Attempts to isolate immune globulins from IgG fractions of antisera by immunoabsorption on Biogel P-300 (using glutaraldehyde linked ligand) were unsuccessful. However, when IgG ligand was bound to cyanogen bromide-activiated Sepharose 4B, an effective absorbent was produced. The following steps were taken to minimize non-immune protein adherence and elution from the gel product: after conjugation, the gel produce was reacted with Blue Dextran 2,000 to block residual basic urethan groups; all chromatographic buffers contained 0.5 M NaCl; and, after the column was equilibrated with antiserum it was washed with 0.1 M veronal acetate saline, pH 8.5. Antibody

was eluted with 0.1 M glycine-HCl buffered saline at pH 2.0, and promptly neutralized with 1.0 M imidazole, pH 8.5. The immunoabsorbed antibody was compared to its antecedent IgG pool by double radial immunodiffusion. There was little or no loss of ligand binding capacity in the eluted immune IgG, which represented a 25 fold purification based on specific activity.

Conjugation

The conjugation and purification procedures that are currently under investigation are designed to separate the conjugate from the impurities of polymerized ferritin, free activated ferritin, and unconjugated immune globulin. A two stage conjugation using glutaraldehyde was chosen to promote an equimolar ratio of ferritin to IgG in the conjugate. After ferritin activation excess glutaraldehyde is removed by passage through Sephadex G-25. The complex misture resulting from the combination of activated ferritin and immune IgG is resolved by passage through two contiguous columns of 10% and 6% agarose, respectively. Studies in progress are directed at assessing the homogeneity and activity of the conjugate by EM and immunoprecipitation. Results can be expected to be directly applicable to determining the participation and role of the several immunoglobulin in gonococcal urethritis, particularly as related to phagocytosis, bacterial adherence, and penetration of epithelial cells. However, since the principal investigator is leaving in July with no replacement in sight, it is probable that the project will have to be terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00009-3 LSD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Investigation of the Streptolysin S-related Cellular Hemolysins of Group A Streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. B. Calandra	Medical Officer	LSD, NIAID
Other:	T. S. Theodore	Research Microbiol.	LSD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Streptococcal Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Because of reassignment (Dr. Calandra to training outside the service) no work was performed on this project and it is terminated.

Publications:

Calandra, G. B., and Oginsky, E. L.: Cellular streptolysin S-related hemolysins of Group A streptoccus C203S. Infect. Immun. 12: 13-28, 1975.

Calandra, G. B., and Theodore, T. S.: Cellular location of streptolysin O. Infect. Immun. 12: 750-753, 1975.

Nugent, K. M., Calandra, G. B., and Theodore, T. S.: The cellular location of alpha hemolysin in Staphylococcus aureus. Infect. Immun. 12: 270-273, 1975.

Calandra, G. B., Whitt, R. S., and Cole, R. M.: The relationship of cellular potential hemolysin in Group A streptococci to extracellular streptolysin S. Infect. Immun. 13: 813-817, 1976.

LABORATORY OF BIOLOGY OF VIRUSES

1976 Annual Report

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Annual Report of the Laboratory of Biology of Viruses
National Institute of Allergy and Infectious Diseases, NIH
July 1, 1975 through June 30, 1976

The Laboratory of Biology of Viruses has under investigation biologic phenomena such as transformation of cells by viruses, regulation of viral mRNA synthesis, mechanisms of DNA replication and synthesis of viral macromolecules. Particular viruses, because of size or their site or mechanisms of replication are particularly suited for the analysis of a specific problem. Nevertheless, it is clear, as major problems are better understood, that biologic phenomena in apparently diverse systems have similar explanations at the molecular level. This is true in the area of viral oncology when adenoviruses and SV40 are compared. With greater understanding, viral latency and viral transformation merge and can be seen to have many common features. Studies which have characterized vaccinia viral messenger mRNA are seen to apply equally to SV40 and adenovirus mRNA and also to cellular mRNA. In spite of the growing unity of the major areas of research, this annual report divides the work of the LBV into four separate areas. These concern molecular studies with (1) Papovaviruses, (2) Poxviruses, (3) Adenoviruses, and (4) Parvoviruses. The division of the report into these four areas represents a scientifically false separation, but is used as a format for this report since it simplifies the presentation of results. A careful reading of the report will reveal the strength that accrues to the laboratory by an exchange of techniques and a sharing of observations in each of these areas.

The results described below are evidence of solid, well grounded advances in each area of research, and report many new findings of major importance in particular areas.

Studies with Papovaviruses.

There is widespread interest in SV40 since it transforms cells, it is capable of producing tumors in vivo when injected into suitable hosts, and is a virus which has one of the simplest compositions. Its viral genome is a double stranded, covalently closed molecule of 3×10^6 daltons, and so it can direct the synthesis of no more than 5 or 6 viral specified proteins. Largely as a result of work carried out in this laboratory there is a high level of understanding of the way in which synthesis of viral RNA and DNA occurs during a lytic cycle of replication.

Previous reports have described studies with the human papovavirus BK (BKV). These studies have been extended to include JC virus, an agent originally isolated from the brain of a patient with progressive multifocal leukoencephalopathy (PML). Sero-epidemiological studies have established that JC virus (JCV) is distinct from human papovavirus BK (BKV) and simian virus 40 (SV40). Supercoiled JCV DNA was prepared from human embryonic glial cells and found to consist of at least 5 distinct species, the largest of which was 90% the size of SV40 DNA (approximately 3.2×10^6 daltons). Nucleic acid hybridization experiments indicated that JCV and SV40 share

11% of their polynucleotide sequences while 25% homology was noted between JCV and BKV DNAs. Further studies demonstrated that the DNA sequences shared by JCV and SV40 are a subset of the sequences shared by JCV and BKV. These shared DNA sequences are common to the genomes of these three primate papovaviruses (Howley, Martin, Takemoto).

The tumor (T) antigens induced by SV40 and BKV have been purified by immunological techniques and their properties have been compared using gel electrophoretic procedures. These studies indicate that both T antigens can be precipitated with homologous and heterologous antisera and co-migrate during electrophoresis in polyacrylamide gels. Experiments are in progress comparing the tryptic peptides derived from each T antigen preparation (Simmons, Martin).

An alternative purification procedure has been developed which yields a single protein band and represents a 180 fold purification of T antigen. This procedure frees the T antigen of nuclease activity and should prove useful in studying the interaction between T antigen and specific viral DNA regions (Barban, May, Chen).

SV40 transcription intermediates (TI) were isolated from SV40-infected BSC-1 cells after treatment of nuclei with sarkosyl. This allowed a separation of viral nucleoprotein complexes and host chromatin. The isolated viral TI contains an endogenous RNA polymerase activity that incorporates labeled uridine triphosphate into acid precipitable material for at least 60 min at room temperature. The polymerase reaction is dependent on exogenous nucleoside triphosphates and is 96% inhibited by low doses of α-amanitin. High concentrations of rifamicin derivative AF/013 have no effect on the transcription activity. This indicates that RNA polymerase II is involved in the transcriptional activity of the complex and that RNA synthesis had been initiated in-vivo.

When the nucleoprotein complexes are sedimented in neutral sucrose gradients and the gradients assayed for polymerase activity, several reproducible peaks of activity are found which sediment faster than 21S SV40 DNA I. The major peak sediments at about 24S. If the TI molecules are labeled by allowing RNA synthesis to occur in the presence of ^{3}H-UTP before sedimentation, labeled RNA is detected as a broad band extending from 21S to more than 40S with a major peak at about 24S. The heterogeneity of the TI sedimentation can be accounted for by the attachment of nascent RNA chains of various sizes to the DNA template.

When the nascent RNA on the TI molecules are treated with RNase A, a fraction of the RNA remains associated with the TI. In pulse chase experiments the fraction of label that remains RNAse resistant and the length of the resistant RNA piece were determined.

At least 50% of the RNA synthesized in this in-vitro system hybridizes to SV40 DNA. Hybridization with fragments of SV40 DNA generated with endonuclease Hind II and III indicates that this RNA is complementary to all regions of the SV40 genome (Shani, Birkenmeier, May, Salzman).

The SV40 transcription complex is being used as a biologic probe to measure transcriptional activity on the viral genome in the cell. Temperature sensitive mutants which fail to replicate SV40 DNA under restrictive temperatures continue late transcription if initiation of transcription is allowed to occur at the permissive temperature. We are now characterizing the transcription complexes in these mutants and determining what factors may regulate the increase of late gene transcription during infection. We are also studying early viral transcription which occurs before the onset of DNA replication, and we are able to extract an enzymatically active complex early in infection. These studies may help in determining what controls the change from early to late viral gene expression during infection (Birkenmeier, May, Salzman).

Overall, the results indicate that we have been able to isolate an SV40 transcriptional complex which can generate RNA in-vitro. By characterizing the DNA, RNA and proteins in the complex we hope to understand mechanisms which regulate the expression of viral genes.

A technique has recently been described in which complementary RNA can hybridize to double stranded DNA by displacing the part of the DNA strand that is identical to this RNA. An "R loop," is generated that can be visualized by electron microscopy. This procedure offers an attractive alternative method for mapping mRNA molecules. We have used this technique with viral mRNA isolated from cells infected with SV40 and linear molecules generated by cleaving SV40 DNA with either Endo R·Bam I or Endo R·Hpa II. Stable RNA-DNA hybrids are formed in 65% formamide but in dilute aqueous solutions, partial displacement of the RNA molecule can occur. Using this procedure, the 5' end of the 16S viral mRNA has been mapped at .95 map units and the 3' terminus of the poly A containing mRNA is located at .20 map units. The 3' end of the viral specified mRNA terminates at the Endo R·Hind BG junction (May, Salzman).

Studies concerning in-vitro SV40 DNA replication have employed several different systems. One goal of the current studies is to determine what common elements are shared by prokaryotic and eukaryotic cells that are concerned with DNA replication. SV40 replicative intermediates which were isolated from infected monkey cells have been used to study the continuation of DNA synthesis in E. coli cell extracts. Although DNA replication was poor, a low level of radioactive precursors has been incorporated into replicative intermediates and a small amount (1-2%) of DNA I has been formed. In the presence of the nuclear extract from nonkey cell, significant quantities of prelabeled 4S DNA from replicative intermediates have been converted into longer strands and a small amount (1-5%) of DNA I has been formed. Characterization of the soluble multienzyme complex responsible for the synthesis of SV40 DNA is under study (Chen, Salzman).

Studies have also been conducted to evaluate unique DNA forms that are present in monkey cells productively infected with SV40. We have detected a series of oligomeric (monomer through hexamer) SV40 DNA molecules that appear during the latter stages of virus infection. At present we are evaluating how these DNA molecules arise, what their role is during productive infection and whether they are important elements in effecting cell transforma-

tion (Martin).

Experiments have been initiated to assess potential biohazards associated with the preparation and use of recombinant DNA molecules. We have perfected methods of ligating specific restriction endonuclease cleavage products together. The ultimate goal is to clone polyoma DNA in E. coli using plasmid and phage vector systems, and test any products made by inoculating mice (adult, newborn, germ-free, conventional) by several different routes (Israel, Martin).

A study is being carried out on the role of single strand DNA binding proteins in the synthesis of SV40 DNA in-vivo and in-vitro. Single strand DNA binding proteins have been described, most notably by the Albert's group, and criteria for their identification and procedure for their purification have been developed. These procedures are being used to identify and analyze such proteins in cells permissive for SV40 growth. The methods include DNA cellulose column chromatography and electrophoresis or SDS acrylamide gels. So far, some 30-50 proteins have been identified as DNA binding proteins. By examination of the pattern of synthesis of these proteins in uninfected stationary phase cells, and growing cells, cells infected with wild type virus, and cells infected with tsA gene viral mutants, it should be possible to identify one or two such proteins that are involved in the synthesis of SV40 viral DNA. These proteins will then be studied to determine if they enhance the synthesis of DNA in-vitro in a system composed of DNA polymerase and appropriate DNA templates (Seidman, Salzman).

Adenoviruses.

Structural characterization of the adenovirus genome has progressed over the past year. A physical map of Ad 18 DNA was obtained by ordering fragments produced by several restriction endonucleases used both separately and sequentially. Specific viral function can now be located on this physical map. Of particular interest is whether the ad 18 genome contains a repetition of oncogenic sequences within its long inverted terminal repetition. If so, a "double dose" of transforming genes would be present and could account for the high oncogenic potency of this virus (Garon, Rose).

In another study insite was gained concerning the mechanism of adenovirus DNA replication. Single-stranded viral DNA representing both parental DNA strands was isolated during synthesis of Ad 2 DNA in infected cells. This single-stranded DNA was eventually incorporated into double-stranded viral genomes, showing that these single strands represent intermediates in the synthesis of viral DNA. Based on a strand displacement mechanism of DNA synthesis, this finding indicates that viral DNA synthesis can be initiated from either end of the parental duplex molecule. The specific initiation process, which may relate to initiation of cellular DNA synthesis, is now being investigated (Lavelle, Rose).

Parvoviruses.

These are the smallest DNA-containing animal viruses and they are divided

into two groups, nondefectives and defectives. The defectives, or AAV, can multiply only when cells are also infected with a helper adenovirus. Parvoviruses contain a single-stranded DNA genome, and, in the case of AAV, DNA replication proceeds via concatemeric intermediates consisting of covalently linked plus and minus strands. These intermediates provide evidence for a self-priming mechanism for the synthesis of AAV DNA. Furthermore, concatemers are processed to unit length molecules in-vivo. Current studies suggest that this specific processing activity can act in-vitro. Such an enzyme has not yet been isolated from an animal cell, and if cellular in origin, it might also play a role in cellular DNA synthesis (Sebring, Rose).

It was shown previously that AAV structural protein consists of three polypeptides, and that the sum of their molecular weights exceeds the coding capacity of the viral genome. The origin of these polypeptides has been studied by using hybridization-selected AAV messenger RNA to direct virus-specific protein synthesis in a cell-free mammalian translating system. It was found that the largest structural polypeptide is viral coded, whereas the remaining two polypeptides are likely derived from the largest protein. The mechanism by which the smaller proteins arise may be of general importance and is currently being investigated (Buller, Rose).

In studies with Kilham rat virus (KRV), a nondefective parvovirus, it was found that 5-6% of the extracted genome is double-stranded. This double-stranded DNA results from a folding over of the ends of the strand to form short hairpin-like terminal regions. The 3'-ended terminus could thus serve to self-prime DNA synthesis in a way similar to that described for AAV. Self-primed DNA synthesis may therefore be a general feature of all parvoviruses (L. Salzman).

In addition, a nucleoprotein complex has been isolated from the KRV virion. The protein from the complex resembles in size and requirements the previously described KRV-associated DNA polymerase. The finding of this complex suggests that the virus-associated enzyme is an integral part of the virion structure and provides further evidence that a structural component may serve a dual purpose (L. Salzman, Koczot).

Poxviruses.

The poxviruses provide the most useful in-vitro system for studying DNA-dependent synthesis of messenger RNA (mRNA). It appears that the majority, if not all of the enzymes involved in transcription are packaged within the core of the infectious virus particle. During the past few years Moss and co-workers have succeeded in dissecting out many of these enzymes. Of great importance was the discovery that vaccinia virus mRNA has a sequence of methylated nucleotides at the 5'-terminal. Three enzymes, two of which were isolated as a complex, are utilized to synthesize this structure which is needed for mRNA to bind to ribosomes. Evidence has been obtained that the modification occurs by a post-transcriptional mechanism in which first a GMP residue is transferred to the 5'-terminal of nascent mRNA, then a methyl group

is added to the base of the added GMP, and finally a methyl group is added to the ribose of the now penultimate nucleoside. These enzymes belong to the class of "early" viral gene products which are synthesized soon after infection of cells. Using the experience obtained with vaccinia virus mRNA, the sequence of methylated nucleotides at the 5'-terminal of other viral and cellular mRNAs have been determined. The enzymatic steps involved in their formation are now being investigated. Continuation of this work is likely to increase our knowledge of how viral and cellular mRNAs are synthesized and processed and may lead to an understanding of the mechanisms used by viruses to regulate cell protein synthesis (Moss, Wei, Ensinger, Martin).

Efforts are being made to obtain a physical and transcriptional map of vaccinia virus DNA, in order to study the organization of genes in large DNA molecules. The first step has been to fragment the DNA into 10 to 16 specific pieces which can be separated on the basis of size. The second step has been to determine which fragments are derived from the ends of the DNA and which from the middle. The latter has been facilitated by the covalent linkage of the complementary strands of vaccinia DNA at the two ends of the molecule. By controlled shearing of the intact genome, fragments 1/4 or 1/8 genome-size have been obtained and the cross-linked end pieces isolated. As anticipated, a much smaller number of restriction fragments were obtained by digestion of the isolated ends (DeFilippes).

Molluscum contagiosum virus, another member of the poxvirus group, is of particular interest because it produces benign tumors of the skin in humans. Electron microscopy of the isolated DNA indicates that it is similar in length to vaccinia DNA and also has the characteristic cross-links, visible as bubbles upon partial denaturation, at the ends of the genome. Restriction endonuclease cleavage of molluscum contagiosum DNA has produced 7 fragments. Electron microscopic attempts are now being made to determine which of the fragments represent the ends of the DNA (Garon).

Honors & Awards

This past year Dr. Moss was appointed an associate editor of Virology and he continues to serve on the editorial boards of the Journal of Virology, Antibiotics and Chemotherapy and Intervirology. Dr. Martin was appointed to the editorial board of the Journal of Virology. Dr. Salzman also continues on the editorial board of the Journal of Virology. Dr. Salzman was reappointed as a professorial lecturer in the Department of Microbiology at Georgetown University School of Medicine.

Annual Report of the Laboratory of Biology of Viruses
National Institute of Allergy and Infectious Diseases
July 1, 1975 through June 30, 1976

Administration

This Spring, Dr. George Khoury left LBV in order to become a section chief in the National Cancer Institute. Dr. Marcia Ensinger and Dr. Scott Martin will leave this Summer, and Dr. Nancy Chiu (visiting fellow), Dr. Ernest Barbosa (research associate), Dr. Jerry Keith (staff fellow), Dr. Peirre May (visiting scientist) and Dr. Tonu Wali (research fellow) will join this Laboratory.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00122-02 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Characterization of Papovavirus Genomes in Lytically Infected and Transformed Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Malcolm A. Martin	Medical Officer (Research)	LBV NIAID
Other:	Mark Israel	Research Associate	LBV NIAID
	Daniel Simmons	Staff Fellow	LBV NIAID
	Peter Howley	Research Associate	LBV NIAID

COOPERATING UNITS (if any)

Kenneth Takemoto, Laboratory of Viral Diseases, NIAID, NIH

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Physical Biochemistry Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	4	4

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been conducted to evaluate the polynucleotide sequence homology between human papovaviruses JC and BK and SV40. JCV shares approximately 25% of its sequences with BKV and 11% with SV40. Experiments are also in progress to examine the relationship bwtween BKV and SV40 tumor antigens and to characterize the structure, mode of synthesis and biological function of oligomeric forms of papovaviruses.

Project Description: Project Number Z01 AI 00122-02 LBV

Our work with human papovaviruses has been extended to include JC virus, an agent originally isolated from the brain of a patient with progressive multifocal leukoencephalopathy (PML). Sero-epidemiological studies have established that JC virus (JCV) is distinct from human papovavirus BK (BKV) and simian virus 40 (SV40). Supercoiled JCV DNA was prepared from human embryonic glial cells and found to consist of at least 5 distinct species, the largest of which was 90% the size of SV40 DNA (approximately 3.2×10^6 daltons). Nucleic acid hybridization experiments indicated that JCV and SV40 share 11% of their polynucleotide sequences while 25% homology was noted between JCV and BKV DNAs. Further studies demonstrated that the DNA sequences shared by JCV and SV40 are a subset of the sequences shared by JCV and BKV. These shared DNA sequences are common to the genomes of these three primate papovaviruses.

We have devoted a considerable amount of time to purifying the tumor (T) antigens induced by SV40 and BKV. To date our studies indicate that both T antigens can be precipitated with homologous and heterologous antisera and comigrate during electrophoresis in polyacrylamide gels. Experiments are in progress comparing the tryptic peptides derived from each T antigen preparation.

Studies have also been conducted to evaluate unique DNA forms that are present in monkey cells productively infected with SV40. We have detected a series of oligomeric (monomer through hexamer) SV40 DNA molecules that appear during the latter stages of virus infection. At present we are evaluating how such DNA molecules arise and what their role is during productive and transforming infection.

Experiments have been initiated to assess potential biohazards associated with the preparation and use of recombinant DNA molecules. We have perfected methods of ligating specific restriction endonuclease cleavage products together. Our ultimate goal is to clone polyoma DNA in E. coli using plasmid and phage vector systems, and test any products made by inoculating mice (adult, newborn, germ-free, conventional) by several different routes.

Key Words:

Papovavirus DNA, physical map, polynucleotide sequence homology, virus transformed cells and integrated viral DNA

Publications:

Khoury, G., Howley, P. M., Garon, C., Mullarkey, M. F., Takemoto, K. K., and Martin, M. A.: An analysis of the homology and relationship between the genomes of papovaviruses BKV and SV40. Proc. Nat. Acad. Sci. U.S.A., 72: 2563-2567 (1975)

Martin, M. A., and Khoury, G.: Integration of DNA tumor virus genomes. In Current Topics in Microbiology and Immunology, 73: 35-65 (1976)

Howley, P., Khoury, G., Byrne, J., Takemoto, K., and Martin, M.: Physical Map of the BK Virus Genome. J. Virol. 16: 959-973 (1975)

Khoury, G., Carter, B., Ferdinand, F., Howley, P., Brown, M., and Martin, M.: Genome Localization of Simian Virus 40 RNA Species. J. Virol. 17: 832-840 (1975)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00123-10 LBV

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure, Synthesis and Function of Viral Macromolecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bernard Moss	Medical Officer (Research)	LBV NIAID
Other:	Cha-Mer Wei	Staff Fellow	LBV NIAID
	Scott A. Martin	Research Associate	LBV NIAID
	Robert F. Boone	Research Associate	LBV NIAID
	Marcia J. Ensinger	Guest Worker	LBV NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	5	3

SUMMARY OF WORK (200 words or less - underline keywords)

The major objectives of this project are to elucidate the roles of viral macromolecules in the replication of viruses and regulation of host processes. A long range goal is to evaluate specific viral functions as targets of antiviral agents. Current studies are focused on the structure, synthesis, modification, and function of messenger RNA (mRNA). We found that both viral and cellular mRNAs contain a sequence of methylated nucleotides at their 5'-terminals, i.e. that end of the mRNA that binds to ribosomes for translation, as well as at internal locations. In the case of vaccinia virus, the mRNA modification enzymes have been isolated and the steps involved in the formation of the 5'-terminal structure have been determined. Similar work on the modification of cellular mRNAs is in progress. We anticipate that continuation of these studies will further our understanding of the synthesis and processing of viral and cellular mRNAs. The possibility that regulation of viral or cellular protein synthesis is mediated by the modification of mRNA is under investigation.

Project Description:

Major Findings:

1. During the previous year we discovered a novel sequence of methylated nucleotides at the 5'-terminals of vaccinia virus and HeLa cell mRNAs. The structure, which may be required for translation of mRNA, consists of a terminal 7-methylguanosine residue linked by a 5' to 5' triphosphate bridge to a 2'-O-methylated ribonucleoside represented as $m^7G(5')ppp(5')N^m$-. Similar sequences have now been identified in mRNAs of other viruses including adenovirus and in specific classes of cellular mRNAs including those that synthesize histones. The penultimate nucleoside in many cases has been found to be a novel dimethylated adenosine (N^6,$O^{2'}$-dimethyladenosine) not previously found in either transfer or ribosomal RNAs.

2. Cellular and some viral messenger RNAs also contain 1 to 3 internal N^6-methyladenosine residues located distal from either end of the RNA. In HeLa cell mRNAs the internal N^6-methyladenosine residue is preceded by an unmethylated adenosine or guanosine residue and followed only by a cytidine residue. This indicates a considerable sequence specificity since there are thousands of different mRNAs in HeLa cells. Nevertheless, the function of the N^6-methyladenosine residues are not known.

3. The mechanism of formation of the 5'-terminal structure of vaccinia virus mRNA has been determined. Two of the three enzymes, mRNA guanylyltransferase and mRNA (guanine-7-)-methyltransferase have been purified to near homogeneity and extensively characterized. The guanylyltransferase catalyzes the transfer of a GMP residue from GTP to a 5'-terminal diphosphate on RNA or synthetic polyribonucleotides; the guanine-7-methyltransferase then catalyzes the methylation of the added GMP. Both enzymes are synthesized following infection of cells and belong to the class of early viral gene products.

4. The enzyme responsible for the methylation of the terminal guanosine residue of HeLa cell messenger RNA has been purified and characterized. The specificity of the guanine-7-methyltransferase indicates that G(5')pppN- structures are intermediates in the modification of cellular mRNA.

5. Vaccinia virus mRNA synthesized in infected cells undergoes further modifications apparently by host cell enzymes. The additional methylations occur on the N^6-position of adenosine residues adjacent to the 5'-terminal and at the 2'-position of the third ribonucleoside. Following these additions, vaccinia viral mRNA closely resembles the mRNA of the host cell.

Significance to Biomedical Research and the Program of the Institute:

The modification of the 5'-terminals of viral and cellular mRNAs is a new and exciting finding. Characterization of the enzymes involved in the formation of the 5'-terminal structure has furthered our understanding of the steps in synthesis and processing of mRNA. Evidence that the terminal 7-methylguanosine

residue is needed for translation of mRNA suggests that this modification might play a key role in regulation of cell and viral protein synthesis.

Proposed course:

1. Purify and characterize the mRNA ribonucleoside-2'-methyltransferase, the remaining vaccinia enzyme involved in 5'-terminal modifications.

2. Isolate all of the cellular enzymes involved in the 5'-terminal modification of mRNA in order to define the steps in processing.

3. Determine whether the 5'-terminal modifications are involved in (a) the turn-off of host protein synthesis following vaccinia virus infection; (b) the regulation of early and late vaccinia protein synthesis; (c) the action of interferon in vaccinia virus-infected cells.

Publications:

Moss, B., Rosenblum, E. N. and Gershowitz, A.: Characterization of a poly-riboadenylate polymerase from vaccinia virions. J. Biol. Chem., 250: 4722-4729 (1975)

Ensinger, M. J., Martin, S. A., Paoletti, E. and Moss, B.: Modification of the 5'-terminus of mRNA by soluble guanylyl and methyl transferases from vaccinia virus. Proc. Nat. Acad. Sci. U.S.A., 72: 2525-2529 (1975)

Moss, B., Ensinger, M. J., Martin, S. A., Wei, C. M.: Modification of the 5'-terminus of mRNA by guanylyl and methyl transferases from vaccinia virus. In Beaud, G. and Haenni, A. L. (Eds.): In vitro transcription and translation of viral genomes. INSERM-EMBO, Paris, pp. 161-168 (1975)

Rosemond-Hornbeak, H. and Moss, B.: Inhibition of host protein synthesis by vaccinia virus: Fate of cell mRNA and synthesis of small poly(A)-rich poly-ribonucleotides in the presence of actinomycin D. J. Virol., 16: 34-42 (1975)

Wei, C. M., Gershowitz, A. and Moss, B.: N^6, $O^{2'}$-dimethyladenosine, a novel methylated ribonucleoside next to the 5'-terminal of animal cell and virus mRNAs. Nature, 257: 251-253 (1975)

Martin, S. A., Paoletti, E. and Moss, B.: Purification of mRNA guanylyltransferase and mRNA (guanine-7-)-methyltransferase from vaccinia virions. J. Biol. Chem., 250: 9322-9329 (1975)

Martin, S. A., and Moss, B.: Modification of RNA by mRNA guanylyltransferase and mRNA (guanine-7-)-methyltransferase from vaccinia virions. J. Biol. Chem., 250: 9330-9335 (1975)

Wei, C. M., Gershowitz, A. and Moss, B.: 5'-terminal and internal methylated nucleotide sequences in HeLa cell mRNA. Biochem., 15: 397-401 (1975)

Moss, B. and Koczot, F.: Sequence of methylated nucleotides at the 5'-terminus of adenovirus-specific RNA. J. Virol., 17: 385-392 (1975)

Moss, B., Gershowitz, A., Wei, C. M. and Boone, R.: Formation of the guanylylated and methylated 5'-terminus of vaccinia virus mRNA. Virology, in press

Ensinger, M. J. and Moss, B.: Modification of the 5'-terminus of mRNA by an RNA(guanine-7-)-methyltransferase from HeLa cells. J. Biol. Chem., in press

Moss, B., Martin, S. A., Ensinger, M. J., Boone, R. F. and Wei, C. M.: Modification of the 5'-terminals of mRNAs by viral and cellular enzymes. In Cohn, W. E. and Volkin, E. (Eds.): Progress in nucleic acid research and molecular biology, Academic Press, New York, in press.

Moss, B.: Poxvirus. In Nayak, D. P. (ed.): The molecular biology of animal viruses, Marcel Dekker, New York, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00124-07 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Replication of the Parvovirus KRV

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Lois A. Salzman Research Chemist LBV NIAID

COOPERATING UNITS (if any)
Dr. Darrell Campbell, Jr., National Cancer Institute, NIH

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

The linear, single-stranded DNA from the parvovirus KRV contains regions of double strandedness. These double-stranded regions contain 230 to 300 nucleotides and involve the 5' and probably the 3' termini. They may be involved in viral DNA replication. A double-stranded form of KRV, DNA, probably an intermediate in DNA replication, is synthesized by the infected cell 4 to 6 hours after infection. The synthesis of double-stranded DNA decreases as virion single-stranded DNA synthesis increases. A nucleoprotein complex has been isolated from KRV virions. The complex contains KRV DNA and a protein with the requirements activity and size of the KRV-associated DNA polymerase.

We have found that rat adenocarcinoma cells 13762 (a DMBA induced tumor) and another rat tumor line (C58NT)D are inhibitory to lymphocyte proliferative responses. This inhibition is due to a contamination of the line with KRV. The presence of such viruses in in vivo passaged tumor lines may adversely affect the measurement of lymphoproliferative responses to tumors in vitro.

Project Description:

The parvoviruses have been found associated with or implicated as the causitive agent in many disease states including several types of malignancies hepatitis A and adult gastroententis. The prototype of the autonomous parvoviruses is the Kilham rat virus (KRV). We feel that information on the interaction of KRV with an infected cell resulting in virus replication and cell lysis may have broad application in the study and control of the virus infected cell.

The DNAs from those parvoviruses which have been studies are single-stranded linear molecules with molecular weights ranging from 1.4 to 2.1 x 10^6. Since little is known about the replication of linear single-stranded DNA we have studied the DNA from the virion and the production of viral DNA in the infected cell. KRV-virion DNA (molecular weight 1.5 to 1.6 x 10^6) was uniformly labeled with the radioisotope ^{32}P and then subjected to digestion by single-stranded DNA specific S_1 nuclease. About 5 to 6% of total radioactivity (230 to 300 nucleotides) is found in an S_1 nuclease resistant fragment. These fragments elute from benzoyl-naphthoyl-DEAE (BND) cellulose as double stranded DNA. Specifically labelling the 5' end of the DNA with radioisotopes or specifically digesting the 3' end with nucleases has shown that the 5' end and probably also the 3' ends of the KRV-DNA have a short double stranded region. The 3' double-stranded DNA termini could serve as a primer for the replication of the linear single-stranded DNA.

Within 1 hour after infection of a cell 30 to 40% of the virion DNA is converted to a double-stranded linear DNA form. The double-stranded (D.S.) linear DNA is synthesized maximally at 4-6 hours after infection. The synthesis of D.S.-DNA decreases by 7 hours after infection when the synthesis of single-stranded virion DNA begins. The double-stranded DNA is believed to be an intermediate in replication. The D.S.-DNA has been shown to contain a DNA strand complimentary to KRV virion DNA by hybridization techniques. We hope to isolate and study further the replicative intermediates. Some of the enzymes required for the conversion of infectious virion single-stranded linear DNA to double-stranded linear DNA to virion linear single-stranded DNA may be unique to the infected cell.

We have isolated a KRV nucleoprotein complex from the virion. If the KRV virion is disrupted at pH 10 and sedimented in an alkaline sucrose gradient a peak of DNA and a larger peak of DNA associated with protein can be isolated. The nucleoprotein complex has a sedimentation of 9-10S. It contained DNA shown by electron microscopy to be the same size and configuration as virion DNA. The protein from the complex resembled in size and requirements the previously described KRV associated DNA polymerase. The finding of the viral nucleoprotein complex suggests that the virus associated enzyme may be an integral part of the virion.

We are also working in the laboratory on transcription of the viral DNA and have worked out the proceedure including oligo dT cellulose chromatography for isolating KRV messenger RNA. The viral specific RNA contains a region of poly A. We have worked out the conditions for cutting the double-stranded KRV replicative intermediate with restriction enzymes and hope to use the DNA fragments for mapping the genome.

In conjunction with Dr. Darrell Campbell (NCI) we have found that rat adenocarcinoma cells 13762 (a DMBA induced tumor) are strongly inhibitory to lymphocyte proliferative responses. This inhibition was due to a contamination of the line with KRV. The virus can replicate in rat lymphocytes, predominately in lymphocytes stimulated to active proliferation. Other transplantable rat tumor lines (C58NT) D is also contaminated with KRV. The presence of such viruses in in vivo passaged tumor lines may adversely affect the measurement of lymphoproliferative responses to tumors in vitro.

Key Words: Viral single-stranded DNA replication, Virus associated DNA polymerases, viral transcription and translation, Parvovirus (KRV).

Publications:

Salzman, L. A. and McKerlie, L.: Nuclear and Cytoplasmic DNA polymerases from rat nephroma cells. J. Biol. Chem. 250: 5589-5595, 1975.

Salzman, L. A. and McKerlie, L.: Characterization of the Deoxyribonucleic acid polymerase associated with Kilham rat virus J. Biol. Chem. 250: 5580-5588, 1975.

Salzman, L. A.: Parvoviruses in Nayak, D. P. (Ed).; Molecular Biology of Viruses. New York, Marcel Dekkev, Inc., 1976.

Salzman, L. A.: Evidence for Terminal Palindromic Sequences on Single-stranded linear DNA. Virology. In press.

Salzman, L. A. and Koczot, F.: Isolation of a Nucleoprotein Complex from the parvovirus, KRV. J. Virol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00125-07 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mechanism of DNA Replication and Integration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman P. Salzman	Chief	LBV, NIAID
Other:	Edward Birkenmeier	Research Associate	LBV, NIAID
	Michael Chen	Staff Fellow	LBV, NIAID
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COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9	6	3

SUMMARY OF WORK (200 words or less - underline keywords)

Characterization of SV40 DNA replicating molecules is being used to determine the mechanism of replication in lytically infected African green monkey kidney cells. Studies are being carried out on the mechanism of chain elongation and to characterize the proteins associated with replicating molecules. Nascent RNA chains that are attached to template DNA molecules are being isolated and regulation of viral m-RNA transcription is being studied.

Project Description:

We have continued our study which seeks to define events during a cycle of replication of SV40 in monkey kidney cells. During the past year greater emphasis has been given to viral m-RNA synthesis in cell free systems. In addition, studies of SV40 DNA replication have continued.

In-vitro Synthesis of Viral m-RNA.

SV40 transcription intermediates (TI) were isolated from SV40-infected BSC-1 cells after treatment of nuclei with sarkosyl. This allowed a separation between viral nucleoprotein complexes and host chromatin. The isolated viral TI contains an endogenous RNA polymerase activity that incorporates labeled uridine triphosphate into acid precipitable material for at least 60 min at room temperature. The polymerase reaction is dependent on exogenous nucleoside triphosphates and is 96% inhibited by low doses of α-amanitin. High concentrations of rifamicin derivative AF/013 have no effect on the transcription activity. This indicates that RNA polymerase II is involved in the transcriptional activity of the complex and that RNA synthesis had been initiated in-vivo.

When the nucleoprotein complexes are sedimented in neutral sucrose gradients and the gradients assayed for polymerase activity, several reproducible peaks of activity are found which sediment faster than 21S SV40 DNA I. The major peak sediments at about 24S. If the TI molecules are labeled by allowing RNA synthesis to occur in the presence of ^{3}H-UTP before sedimentation, labeled RNA is detected as a broad band extending from 21S to more than 40S with a major peak at about 24S. The heterogeneity of the TI sedimentation can be accounted for the attachment of nascent RNA chains of various sizes to the DNA template.

When the nascent RNA on the TI molecules are treated with RNase A, a fraction of the RNA remains associated with the TI. In pulse chase experiments where the fraction of label that remains RNAse resistant and tbe length of the resistant RNA piece were determined, the rate of RNA chain growth could be calculated.

At least 50% of the RNA synthesized in this in-vitro system hybridize to SV40 DNA. Hybridization with fragments of SV40 DNA generated with endonuclease Hind II and III indicates that this RNA is complementary to all regions of the SV40 genome.

The SV40 transcription complex is being used as a biologic probe to measure transcriptional activity on the viral genome in the cell. Temperature sensitive mutants which fail to replicate SV40 DNA under restrictive temperatures continue late transcription if initiation of transcription is allowed to occur at the permissive temperature. We are now characterizing the transcription complexes in these mutants and determining what factors may regulate the increase of late gene transcription during infection. We are also studying early viral transcription which occurs before the onset of DNA

replication, and we are able to extract an enzymatically active complex early in infection. These studies may help in determining what controls the change from early to late viral gene expression during infection.

Overall, the results indicate that we have been able to isolate an SV40 transcriptional complex which can generate RNA in-vitro. By characterizing the DNA, RNA and proteins in the complex we hope to understand mechanisms which regulate the expression of viral genes.

Mapping of Viral m-RNA Transcripts.

A technique has recently been described in which complementary RNA can hybridize to double stranded DNA by displacing the part of the DNA strand that is identical to this RNA. An "R loop," is generated that can be visualized by electron microscopy. This procedure offers an attractive alternative method for mapping m-RNA molecules. We have used this technique with viral m-RNA isolated from cells infected with SV40 and linear molecules generated by cleaving SV40 DNA with either Endo R·Bam I or Endo R·Hpa II.

Stable RNA-DNA hybrids are formed in 65% formamide but in dilute aqueous solutions partial displacement of the RNA molecule can occur. Using this procedure, the 5' end of the 16S viral m-RNA has been mapped at .95 map units and the 3' terminus of the poly A containing m-RNA is located at .20 map units. The 3' end of the viral specified m-RNA terminates at the Endo R·Hind BG junction.

In-vitro Synthesis of SV40 DNA in Soluble Systems.

Studies concerning in-vitro SV40 DNA replication have employed several different systems. One goal of the current studies is to determine what common elements are shared by prokaryotic and eukaryotic cells that are concerned with DNA replication. SV40 replicative intermediates which were isolated from infected monkey cells have been used to study the continuation of DNA synthesis in E. coli cell extracts. Although DNA replication was poor, a low level of radioactive precursors has been incorporated into replicative intermediates and a small amount (1-2%) of DNA I has been formed. In the presence of the nuclear extract from monkey cell, significant quantities of prelabeled 4S DNA from replicative intermediates have been converted into longer strands and a small amount (1-5%) of DNA I has been formed. Characterization of the soluble multienzyme complex responsible for the synthesis of SV40 DNA is under studies.

Role of DNA Binding Proteins in SV40 DNA Synthesis.

A study is being carried out on the role of single strand DNA binding proteins in the synthesis of SV40 DNA in-vivo and in-vitro. Single strand DNA binding proteins have been described, most notably by the Albert's group, and criteria for their identification and procedure for their purification have been developed. These procedures are being used to identify and analyze such proteins in cells permissive for SV40 growth. The methods include DNA

cellulose column chromatography and electrophoresis or SDS acrylamide gels. So far, some 30-50 proteins have been identified as DNA binding proteins. By examination of the pattern of synthesis of these proteins in stationary phase cells (uninfected), growing cells, cells infected with wild type virus, and cells infected with tsA gene viral mutants. It should be possible to identify one or two such proteins that are involved in the synthesis of SV40 viral DNA. These proteins will then be studied to determine if they enhance the synthesis of DNA in-vitro in a system composed of DNA and polymerase and appropriate DNA templates.

Key Words:

viral m-RNA synthesis, cell-free systems, transcription intermediates, exogenous nucleoside triphosphates, nascent RNA chains, "R loop", Endo R·Bam I, Endo R·Hpa II, prokaryotic and eukaryotic cells, DNA cellulose column chromatography, SDS acrylamide gels.

Publications:

Zweig, M., Barban, S. and Salzman, N. P.: Analysis of simian virus 40 wild-type and mutant virions by agarose gel electrophoresis. J. Virol. 17: 916-923, 1976.

Chen, M., Birkenmeier, E. and Salzman, N. P.: Simian virus 40 DNA replication: Characterization of a gap at the DNA termination site. J. Virol. 17: 614-621, 1976.

Chen, M., Lebowitz, J. and Salzman, N. P.: HINd restriction mapping of unpaired regions in SV40 superhelical DNA I. J. Virol. 18: 211-217, 1976.

Lebowitz, J., Garon, C. F., Chen, M. C. Y. and Salzman, N. P.: Chemical modification of SV40 DNA by reaction with a water-soluble carbodiimide. J. Virol. 18: 205-210, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00126-03 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Restriction Enzyme Analysis of Vaccinia Virus DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Frank DeFilippes Research Physicist (Gen) LBV NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to study the organization of genes in large DNA molecules obtained from animal viruses. The production and ordering of unique segments of vaccinia virus DNA provides a physical map of the genome. A transcriptional map of the genome can also be determined by use of the isolated unique DNA segments. The identification of blocks of genes which produce functionally related proteins is obtained by examining transcription, and translation of the RNA products of DNA segments larger than 10^7 daltons. The preparation of several different restriction enzymes which cut vaccinia DNA into these large segments is necessary. I am also working to improve the techniques for separating and isolating very large pieces of DNA which differ in size and base composition by relatively small amounts.

Vaccinia DNA has covalently-linked complementary (CLC) ends which serve as reference points in the physical map of the genome. Controlled shearing of the intact DNA can be used to produce fragments which are about 1/2 or 1/4 or 1/8 of the original length. If the sheared fragments are gently denatured at alkaline pH and the solution neutralized, then the end fragments rapidly renature and they can be separated from the single-stranded DNA, originating in the interior of the molecule, by chromatography on BND-cellulose. The double-stranded fragments serve as the substrate for a restriction enzyme that cleaves the DNA into specific segments which are separated by gel electrophoresis. The electrophoretic pattern, which is characteristic for each enzyme, becomes simpler as the complexity of the DNA substrate is decreased. The shearing process and BND-cellulose chromatography is used to simplify the electrophoretic pattern. Direct digestion of intact vaccinia DNA with the restriction enzyme from Streptomyces albus produces about 16 segments, with the largest 2 segments representing over one-third of the genome. Shearing the DNA at 5000 rpm removes the gel bands with the 2 largest DNA segments as well as 6 other bands. These segments are in the middle of the DNA. Shearing at 7500 rpm removes 3 more bands, leaving the 5 bands with DNA adjacent to the ends. If the entire restriction enzyme digest is denatured and neutralized then 2 bands remain in the gel pattern. The restriction enzyme segments which are the ultimate end pieces are relatively small, both being below 10^7 daltons. The shearing and digestion procedure provides a rough map of the DNA indicating how the segments are arranged around the center of the DNA molecule.

Other restriction enzymes from Serratia marcescens and Xanthomonas malvacearum cleave vaccinia DNA into less than 10 pieces and the end fragments are greater than 10^7 daltons. One of these enzymes cleaves the DNA and leaves a 4 base single-strand tail which makes it useful for genetic engineering experiments.

A fourth restriction enzyme from Bacillus amyloliquefaciens cuts vaccinia DNA into more than 20 pieces. The restriction enzyme DNA segments are isolated and hybridized to vaccinia virus mRNA to obtain a transcriptional map of the genome. Of particular interest is the map obtained with in vitro mRNA which is synthesized in large quantities from vaccinia virus cores. This RNA hybridizes to more than 40% of the equivalent of a single-strand length of vaccinia DNA. However, nothing is known of the relative abundance of the different RNA species present or their origin on the genome.

Vaccinia DNA does not contain a large number of repetitive sequences. Renaturation of sheared DNA fragments indicate that the complexity of the genome is consistent with its molecular weight and is comparable with the complexity of the DNA from T4 bacteriophage which has a similar size and base composition. This indicates that all parts of the DNA should produce unique mRNAs. However, the gel patterns indicate that the segments at each end of the genome may not be unique.

Publications:

DeFilippes, F. M.: Restriction Enzyme Digests of Rapidly Renaturing Fragments of Vaccinia Virus DNA. J. Virol., 17: 227-238 (1976).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00127-09 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Structure and Function of Genetic and Protein Components of Defective Parvoviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Marshall Bloom	Medical Officer	LBV NIAID
	Edwin Sebring	Research Chemist	LBV NIAID
	Frank Koczot	Research Biologist	LBV NIAID
	Claude Garon	Research Microbiologist	LBV NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	3

SUMMARY OF WORK (200 words or less - underline keywords)

Main objectives in studying these defective human parvoviruses (AAV) are to (i) define specific biochemical mechanisms used in synthesizing their DNA, RNA and proteins, (ii) identify and characterize the helper virus-mediated step(s) required for their replication and (iii) relate biochemical findings to normal and altered cellular processes as well as to potentials for selective interference with both AAV and helper virus (adenoviruses, herpesviruses) infection. Among the methods used are nucleic acid hybridizations, analytical and preparative sucrose and CsCl sedimentations, molecular cleavage with restriction nucleases, gel electrophoresis and electron microscopy.

(A) To determine the origin and mechanism of production of AAV structural polypeptides, highly purified AAV messenger RNA (hybridization selected) was used to direct the synthesis of virus-specific protein in a cell-free mammalian translating system. Major findings indicate (i) the largest viral structural polypeptide is viral coded and (ii) the remaining two viral structural polypeptides are likely derived from the largest protein: studies are in progress to elucidate the mechanism by which these latter proteins arise.

(B) We have shown previously that AAV DNA replication proceeds via concatemeric intermediates, and that these intermediates are processed to unit length moleucles in vivo. Current studies directed at identifying and isolating a specific processing enzyme(s) suggest the action of such an activity in vitro. Other studies are aimed at further analyzing the viral DNA replication mechanism, and a system has been developed for the synthesis of AAV DNA in isolated nuclei. Thus far, our overall studies are compatible with a self-priming mechanism for AAV DNA synthesis: it is possible that this mechanism is more general, relating perhaps to eukaryotic cell DNA synthesis.

The molecular biology of AAV is revelant to understanding (i) natural modes of viral infection, (ii) biochemical processes in normal cells and (iii) possible approaches to the treatment of viral disease. We propose to continue studies of AAV replication and defectiveness.

Publications:

Straus, S. E., Ginsberg, H. S., and Rose, J. A.: DNA minus Temperature-sensitive Mutants of Adenovirus Type 5 Help Adenovirus-associated Virus Replication. J. Virol., 17: 140-148 (1976).

Straus, S. E., Sebring, E. D., and Rose, J. A.: Concatemers of Alternating Plus and Minus Strands are Intermediates in AAV DNA Synthesis. Proc. Nat. Acad. Sci., 73: 742-746 (1976).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00128-09 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Properties of Adenovirus DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Claude F. Garon	Research Microbiologist	LBV NIAID
	James A. Rose	Medical Officer	LBV NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

The application of physical and biochemical techniques to identify specific genetic regions in DNAs from both oncogenic and non-oncogenic human adenoviruses, with the ultimate goal of relating these sequences to those biochemical activities which permit these viruses to influence or gain control of cellular functions.

The major objective has been to characterize the molecular structure of both oncogenic and non-oncogenic human adenovirus genomes and to relate specific regions of the genome to the biochemical activities which permit these viruses to influence or gain control of the cells they infect.

Methods used include electron microscopic visualization of macromolecules, sucrose gradient sedimentation analysis, agarose and polyacrylamide gel electrophoresis, restriction endonuclease cleavage mapping and column chromatography on hydroxyapatite and benzalated-naphthalated DEAE cellulose. Major findings include: 1) The first direct, visual evidence for the arrangement of sequences in the inverted terminal repetition of adenovirus DNA. The sequences are responsible for the closure of single-stranded, circular DNA molecules by a duplex, antiparallel, hydrogen-banded projection. 2) The generation of a physical map of Ad 18 DNA by ordering of fragments produced by several restriction endonucleases used both separately and sequentially. The ability to obtain both physically and genetically defined DNA segments is an important prerequisite to the precise mapping of functions involved in viral replication or carcinogenesis or both. 3) Identification of multiple sets of tandemly arranged, complementary sequences which are structurally distinguishable from the sequences within the inverted terminal repetition and are characterized by their extremely rapid, concentration independent reannealing properties. These sequences may be purified as a result of their rapid reannealing properties and their positions within the genome mapped using restriction endonuclease fragments. These sequences may represent important control elements in the expression of the adenovirus genome.

We propose to continue structural analyses of the DNA of both oncogenic and non-oncogenic human adenoviruses with the ultimate goal of relating specific regions of the genome to specific biochemical functions. This information should enhance our understanding of both lytic and latent interactions between this group of viruses and the cells they infect.

Publications:

Garon, C. F., Berry, K. W., and Rose, J. A.: Arrangement of Sequences in the Inverted Terminal Repetition of Adenovirus 18 DNA. Proc. Nat. Acad. Sci. 72: 3039-3043 (1975).

Lavelle, G., Patch, C., Khoury, G., and Rose, J. A.: Isolation and Partial Characterization of Single-stranded Adenoviral DNA Produced During Synthesis of Adenovirus Type 2 DNA. J. Virol., 16: 775-782 (1975).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00129-06 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Biochemical Studies of the Proteins in SV40 Infected and Transformed Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stanley Barban	Research Chemist	LBV NIAID
Other:	Michael Chen	Staff Fellow	LBV NIAID
	Evelyne May	Visiting Associate	LBV NIAID

COOPERATING UNITS (if any)

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Laboratory of Biology of Viruses

SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objectives of studying the proteins of SV40 are: (1) to isolate and characterize all the gene products of the viral genome with the aim of elucidating their roles in viral replication and interaction with the host cells; (2) currently to isolate and characterize the early tumor antigen from a transformed cell line to determine its biological role in establishing and maintaining the transformed state.

During the past year, intensive effort has been applied to isolating and purifying one of the early proteins found in both SV40 lytically infected and transformed cells, the tumor antigen. This antigen is believed to be involved in SV40 DNA replication and may also function in the initiation of cell transformation by the virus. The tumor antigen was extracted from the nuclei of SV40 transformed mouse cell lines and purified by cesium chloride equilibrium centrifugation and chromatography on hydroxyapatite and DNA-cellulose. A preparation of ^{125}I-labeled tumor antigen appeared nearly homogenous as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Starting from the whole cell the purification was calculated to be over 180 fold. The antigen has a strong affinity for double stranded viral DNA and does not have any endonuclease activity. The molecular weight of the protein was determined by sucrose gradient centrifugation and acrylamide gel electrophoresis to be approximately 60,000. Our ultimate goal is to characterize the antigen biochemically and determine its biological role in viral DNA initiation and transformation.

Key Words: SV40 Virus-Proteins, SV40 Tumor Antigen, SV40 Protein-Nucleic Acid Interaction

Publication

Zweig, M., Barban, S., and Salzman, N. P.: Analysis of Simian 40 Wild-Type and Mutant Virions by Agarose Gel Electrophoresis. J. Virol. 17, 916-923, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00156-01 LBV

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structural Characterization of DNA Virus Genomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Claude F. Garon Research Microbiologist LBV NIAID

COOPERATING UNITS (if any)

Dr. J. W. Burnett, University of Maryland Hospital, Baltimore, Maryland

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of these studies has been, using both physical and biochemical techniques, to examine the unique structural features of the Molluscum contagiosum virus genome and to relate these features to the biochemical events involved in the replication and growth of this virus in the cells they infect. Such studies are facilitated by the production of both physically and genetically defined fragments of viral DNA.

The objective of these studies has been to examine the unique structural features of the genomes of several DNA virus groups and to relate these features to the biochemical events involved in the replication and growth of these viruses in the cells they infect. Molluscum contagiosum virus (MCV), a member of the poxvirus group, causes a benign tumor of the skin in humans. In order to obtain detailed information on the genome of this virus, virions were isolated from clinically typical lesions, the DNA extracted and characterized using techniques of electron microscopy, agarose gel electrophoresis, and restriction endonuclease cleavage mapping. Major findings include: I) The genome of MCV appears in the electron microscope as a linear, duplex DNA molecule with a molecular weight of 120 million daltons based on contour length measurement data. However, following complete denaturation of the DNA, single-stranded circles measuring 1.95 times the length of linear, duplex molecules were observed. The MCV genome, therefore, appears to contain two naturally-occurring cross-links at or very near each end of the linear duplex molecule. II) Partial denaturation produced small, single-stranded loops at the ends of all molecules before any internal areas began to denature. The symmetrical appearance of these areas of denaturation might suggest a similar G+C content at each end of the molecule. It might be considered functionally important for the ends of such a viral genome to be the most easily denaturable regions in the molecule. III) When MCV DNA was cleaved by the Bacillus restriction endonuclease BAM·HI, 7 fragments were produced as determined by agarose gel electrophoresis. End fragments may be identified by eluting fragments from the agarose gel and subjecting them to the mounting conditions which produce only the end denaturation pattern. We propose to continue structural analyses of these and other intact viral genomes with the goal of producing physically and genetically defined DNA segments. The production of such fragments is an important prerequisite to direct genetic comparisons among related viral genomes as well as the precise mapping of the biochemical functions involved in viral replication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00121-01 LBV

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Expression of Viral DNA during Lytic and Transforming Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Physical Biochemistry Section

INSTITUTE AND LOCATION
NIH, NIAID, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

LABORATORY OF MICROBIAL IMMUNITY

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PHS-NIH
SUMMARY STATEMENT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
July 1, 1975 - June 30, 1976

Richard Asofsky, M.D.
Chief, Laboratory of Microbial Immunity

Studies in this Laboratory concern basic mechanisms controlling cellular and humoral immunity to microbial and tissue antigens. A wide range of microbial antigens, including several viruses, two blood parasites, and a pneumococcal polysaccharide are used in this work. Tissue antigens are studied in graft-versus-host and conventional (skin) allograft reactions. The newest findings in basic immunology are applied to this work; at the same time, findings in the Laboratory contribute to basic knowledge, such as the description of functional heterogeneity among thymus derived cells (T-cells), the identification of suppressor T cells, and this year the analysis of an immune response under complex genetic control.

COMPLEMENTARY ACTION OF IMMUNE RESPONSE GENES.

Despite the fact that the antibody response to Type III pneumococcal polysaccharide (SSS-III) is almost monoclonal in mature, an analysis of the strain distribution patterns for recombinant - inbred mice (derived from BALB/c, a high responder, and C57Bl/6, a low responder), immunized with this antigen revealed several distinct forms of polygenic control. At least 3-5 genes influence the capacity of bone marrow-derived precursors of antibody-forming cells (B cells) to make a normal antibody response; at least 2 genes control the expression of thymus-derived (T) suppressor activity; and at least 3-4 genes govern amplifier function. These autosomal immune response genes were found to act independently and in a complimentary manner to produce the effects observed; indeed a cross between a low-responding RI strain and low responder C57Bl/6 gave high responses. Although these genes were not found to be associated with genes within the major histocompatibility (H-2) or immunoglobulin allotype (IgC_H) complexes, they could nevertheless influence the expression antibody of responses linked to these genetic loci. (Drs. Baker, Prescott, Ms. Amsbaugh and Mr. Stashak).

PHENOTYPIC DIFFERENCES BETWEEN RECIPROCAL HYBRID MICE.

Reciprocal hybrid mice are derived my mating females from two inbred strains with a male from the alternate strain. Such mice are genotypically identical, except for differences that may be controlled by the Y chromosome of the males. Phenotypic differences were detected in (BALB/c♀ X C57Bl/6♂) (CXB) F_1 mice as compared to (C57Bl/6♀ X BALB/c♂) (BXC) F_1 mice. Spleen cells from post partum BALB/c females which had borne CXB litters but not those that had borne BALB/c litters gave increased GVH reactions in CXB but not in BXC newborn mice as judged by the Simonsen spleen weight assay. Cells from virgin females did not differ in reactivity from cells from postpartum

mothers of BALB/c animals. Postpartum mothers of CXB litters rejected CXB skin in second set fashion; more rapidly than BXC skin and more rapidly than CXB skin was rejected by virgin BALB/c females. (Drs. Herrod, Asofsky, LMI, NIAID; Dr. L. Klassen, NIAMDD).

CELLULAR REQUIREMENTS FOR RESISTANCE TO MALARIA.

The cellular requirements for resistance of mice to a blood transmitted malaria infection that is usually nonlethal in immunocompetent hosts was investigated using T and B cell deficient BALB/c mice. After infection with 17XNL Plasmodium berghei yoelii, both T cell deficient (nude) and B cell deficient (mice injected from birth with anti-μ; μ suppressed) BALB/c mice demonstrated markedly increased parasitemia and mortality compared to appropriate controls (thymus grafted nudes; mice injected with a control hyperimmune anti-ferritin goat antiserum). Thus both T and B cells were required for resistance to a nonlethal malaria infection in BALB/c mice. In contrast, an investigation of the cellular requirements for the induction of anti-sporozoite immunity with irradiated P. berghei sporozoites revealed that although immunization was not effective in T-cell deprived (adult thymectomized, irradiated, bone marrow reconstituted, and ATS treated) C57Bl/6N X BALB/cAnN ($BLCF_1$) mice it was effective in the majority of B-cell deprived (μ-suppressed) $BLCF_1$ mice. This suggested a preimmenent role for T-cell immunization in anti-sporozoite immunity. (Dr. Weinbaum, Dr. Tigelaar, Mr. Evans, LMI; Dr. Chen, LPD).

IN VITRO RESPONSE OF SUBPOPULATIONS OF HUMAN LYMPHOCYTES TO PARASITIC ANTIGENS.

Peripheral blood lymphocytes were fractionated into thymus dependent (T) cells and thymic independent (B) cells. The method of fractionation is a two stage procedure. First, cells are separated into subpopulations highly enriched for either T cells or B cells using an anti-immunoglobulin column. Further purification was obtained by rosetting each subpopulation with sheep erythrocytes. Cells prepared in this manner were highly purified as judged by both mitogen responsiveness and surface fluorescence. Unfractionated cells and T cells were stimulated and proliferated in the presence of antigens prepared from Plasmodium falciparum malaria, Leishmania donovani and Leishmania tropica parasites. B cells did not proliferate in the presence of these antigens. (Drs. Herrod, Weinbaum, Tigelaar, LMI, Drs. Weintraub and Wyler, LPD).

MODULATION OF IMMUNE RESPONSES TO MALARIA.

Immunization against the blood stages of malarial parasites is not yet practical. Mice immunized against Plasmodium berghei showed T-cell activity for at least 6 weeks after immunization, as demonstrated by a delayed footpad swelling (DFS) technique. This response could be augmented by the administration of alkylating agents, in particular 1-bis(2-chloroethyl)-amino-1-oxo-2-aza-5-oxaphosphoridin. Further, the drug was found to modulate the immune response of mice infected with lethal P. berghei. Treated mice demonstrated increased DFS, clearance of parasites, whereas untreated mice evidenced little DFS, and succumbed to infection. This study emphasizes that host immunity can be manipulated by the use of chemical agents in a situation where immunization fails to protect the host. (Dr. Finerty).

IMMUNIZATION OF MICE TO PNEUMOCOCCAL POLYSACCHARIDE SSS-III WITH PURIFIED SAFFLOWER POLYSACCHARIDE.

Mice were immunized with 0.005 µg, 0.05 µg, 0.5 µg or 50 µg of safflower polysaccharide; then, they were assayed (6 days later) for the presence of antibody-producing plaque-forming cells (PFC) making antibody specific for Type III pneumococcal polysaccharide (SSS-III). Here, sheep erythrocytes sensitized with purified SSS-III were used as indicator cells for the detection of PFC. The results obtained showed that mice immunized with these doses of safflower polysaccharide produced 5, 37, 387, 866 and 335 PFC/spleen, respectively. This suggests a fair degree of cross-reactivity between both antigens and the possibility that immunization with safflower polysaccharide may confer significant immunity to SSS-III. Although it is known that many polysaccharide antigens are ubiquitous in nature, this is the first demonstration that a plant polysaccharide can produce immunity to a "type-specific" bacterial antigen. (Dr. Prescott, Mr. Caldes, Dr. Baker).

DISSECTION OF THE DIFFERENTIATION OF THYMUS-DERIVED CELLS (T-CELLS) WITH AN INFECTIOUS AGENT.

The thymic agent (TA), a herpes-like virus of mice, causes almost complete but transient necrosis of the thymus when given to neonatal BALB/c mice. The necrosis is worst at 7-10 days of age; regeneration is nearly complete by 28-35 days of age. T-cell functions were for the most part absent or very greatly reduced during the period of necrosis. These included: helper function for an *in vitro* anti sheep red blood cell response; *in vitro* reactivity to the mitogens PHA and Concanavalin A; graft-versus-host (GVH) activity of spleen and thymus cells; and the capacity of lymph node cells to amplify the GVH reactivity of thymocytes. Two T cell functions were spared: (1) development of suppressor T cells for the antibody response to SSS-III pneumococcal polysaccharide, and (2) directly measured GVH activity of lymph node cells. In the case of the latter activity, normal ontogenetic increase in activity was seen during the period of necrosis. The studies indicate that different T-cell functions are mediated by different kinds of T-cells, some susceptible and some not, to infection with TA; and that GVH activity of lymph node T cells can develop in the absence of functional thymocytes and spleen cells. (Drs. Morse, Asofsky, Baker, LMI; Dr. Mosier, LI, NIAID and Dr. S. Cross, Medical College of Virginia).

IN VITRO STUDIES OF A MOUSE MYELOMA WHICH PRODUCES TWO PARAPROTEINS.

SAMM 368 is a transplantable myeloma which produces an IgA and an IgG2b paraprotein. Tumor cells were established in continuous *in vitro* passage, and paraprotein production examined in three ways: (1) staining of cells with class-specific, affinity column purified antibodies, conjugated either with fluorescein or rhodamine, and examination by fluorescence microscopy. Normally slides were doubly stained; e.g., with a fluorescein conjugated (green) anti IgA and a rhodamine conjugated (red) anti IgG2. Usually all combinations of specificity were used. (2) Incorporation by cultured cells of C14 labeled lysine and isoleucine into immunoglobulins as detected by radio-immunoelectrophoresis using immunoglobulin class specific antisera, and (3) Transplantation of cultured cells into mice, and examination of

serum or ascites for myeloma proteins by immunoelectrophoresis, using immunoglobulin class specific antisera. Of five tissue culture lines established for 1 year, three produce both paraproteins, one produces IgG2b only, and one produces IgA only. Thirty-five clones were obtained from one of the double producers; each was a double producer. The study shows that production of 2 immunoglobulins by individual cells or their progeny can be highly stable. (Dr. Neiders (Guest Worker), Asofsky, Morse, LMI, NIAID, Dr. A. R. Lawton III, University of Alabama).

ADMINISTRATIVE:

The Laboratory was joined this year by Dr. Bonnie Mathieson, a Guest Worker funded by The Cancer Research Institute, Inc., Dr. Francis Nkrumah, officially a Guest Worker in NCI, who works full time with Drs. Tigelaar and Weinbaum, and by two Visiting Fellows, Dr. Colette Kanellopoulos from France and Dr. Kyung-Jin Kim from Korea. Dr. Philip R. B. McMaster left to join the Clinical Pathology Branch of The Clinical Center, for 2 or 3 years residency training. Dr. McMaster maintains experimental animals in the Laboratory's animal quarters, and continues to do research as part of long-standing collaborations with several investigators. The lack of flexibility of space, mentioned last year, has been more serious this year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00133-09 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Molecular Configuration in Immunogenicity, Tolerogenicity and the Allergic Response..

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. R. B. McMaster, M.D.

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

Project terminated in NIAID and transferred with Dr. McMaster to The Clinical Center.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00134-14 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Control of Immunoglobulin Synthesis in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Asofsky	Laboratory Chief	LMI/NIAID
OTHER:	M. E. Neiders	Guest Worker	LMI/NIAID
	H. C. Morse III	Senior Investigator	LMI/NIAID
	A. R. Lawton III*		
	P. S. Campbell	Chemist	LMI/NIAID
	W. J. Logan	Biological Lab. Tech.	LMI/NIAID
	M. Potter	Head, Biochemistry Sec.	LCBGY/NCI
	J. Pumphrey	Biologist	LCBGY/NCI

COOPERATING UNITS (if any)

*University of Alabama School of Medicine, Department of Pediatrics, Birmingham, Alabama 35233.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
32/12	8/12	24/12

SUMMARY OF WORK (200 words or less - underline keywords)

SAMM 368, a transplantable myeloma of BALB/c mice was established in long term tissue culture. The myeloma tumor produces two paraproteins in vivo, an IgA and an IgG2b. Five established culture lines were examined. Three produced both paraproteins, one produced only the IgA, and one produced only the IgG2b. Thirty-five clones of one of the double producers were all double producers.

PHS-6040
(12-75)

Project Description:

(1) Work this year was mostly devoted to establishment in vitro and analysis of two myelomas which produce two paraprotein products, SAMM 368 and TEPC 609 (See: Z01 AI 00135-02 LMI).

The stability of immunoglobulin production of tissue culture lines derived from two BALB/c myelomas producing two classes of immunoglobulins were studied over a period of eleven months. Three methods were used to detect paraprotein synthesis: (a) injection of the tissue culture lines into mice, and examination of serum or ascites with class specific antisera (b) autoradiography of immunoelectrophoretic patterns of supernatants from tissue culture cells cultured in vitro with medium containing C^{14} labelled lysine and isoleucine, (c) staining of tissue culture cells with rhodamine and/or fluorescein labelled purified class-specific antibodies to mouse immunoglobulins. Lines derived from TEPC 609 maintained the production of both immunoglobulins. Three lines out of five established from SAMM 368 maintained production of both IgA and IgG2b, while two lines produced either IgA or IgG2b.

Cloning techniques of the cultured mouse myeloma cells were developed using 0.3% agarose in RPMI 1640 medium with 20% horse serum, non-essential amino acids, and 5×10^{-5} M 2-mercaptoethanol. A feeder layer of oil-induced macrophages or normal spleen macrophages from syngeneic mice was essential for clone production from single cells. The cloning efficiency of the developed technique ranged from 15 to 35 percent using $4\text{-}16 \times 10^{3}$ cells per 60 mm petri dish. The production of immunoglobulins by single cells from SAMM 368 lines producing both immunoglobulins was evaluated by selecting random clones and propagating them in tissue culture. The individual clones were cultured in progressively increasing sizes of tissue culture dishes until each clone reached a number of $2\text{-}3 \times 10^{6}$ cells. The immunoglobulin production of each clone was examined by autoradiography following incorporation of radioactive amino acids or by reinjection of the cells in mice. Using autoradiography 35 clones examined produced both immunoglobulins. Six out of the seven clones giving rise to tumors in mice were producing both immunoglobulins when the resulting ascites was examined by immunoelectrophoresis. One clone was a non-producer.

The studies up to date demonstrate that myeloma producing two classes of immunoglobulins maintains the production of both immunoglobulins in vitro, but occasionally may give rsie to lines that produce a single myeloma protein or lines that loose the production of immunoglobulin.

Experiments are planned that will further characterize the myelomas that produce two immunoglobulins using the presently available lines in tissue culture as well as other newly established lines. This will include:

(1) chromasome analysis to determine whether myelomas producing two immunoglobulins arise by fusion;

(2) studies on isolated single producing variants to determine whether loss of one immunoglobulin is a genetic deletion or an environmental modulation, and

(3) studies on the biosynthesis and assembly of immunoglobulins os myelomas producing two immunoglobuins and their single immunoglobulin producing variants.

(2) Work from last lear on the separation of B and T lymphocytes from mouse spleen was plagued by technical difficulties. Little progress was made, in consequence. However, the same methods have been used for functional fractionation of human blood lymphocytes (See: Z01 AI 00153-01 LMI).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00135-02 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Properties of Immunoglobulin Secreting Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. C. Morse III	Senior Investigator	LMI/NIAID
OTHER:	R. Asofsky	Laboratory Chief	LMI/NIAID
	J. H. Goode	Biologist	LMI/NIAID
	M. Potter	Head, Immunochemistry Section	LCBGY/NCI
	J. G. Pumphrey	Biologist	LCBGY/NCI
	R. Lieberman	Senior Investigator	LI/NIAID
	A. R. Lawton*	Associate Professor	
	M. Weigert**		

COOPERATING UNITS (if any)

* Department of Pediatrics, University of Alabama in Birmingham, Birmingham, Alabama.

** Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
18/12	6/12	12/12

SUMMARY OF WORK (200 words or less - underline keywords)

Three murine plasmacytomas have been found which simultaneously secrete two classes of immunoglobulin. SAMM 368 secretes IgA and IgG2b paraproteins which do not share idiotype. TEPC 609 secretes IgA and IgG2b paraproteins which share idiotype. Both these tumors are from BALB/c mice. A third tumor, 2834, from an NZB mouse secretes IgA and IgG3 paraproteins; studies of the products of this tumor line are in progress.

PHS-6040
(12-75)

Project Description:

These studies were designed to evaluate the mechanisms regulating immunoglobulin gene expression in immunoglobulin producing cells.

A study of the immunoglobulin products of 778 consecutive primary plasmacytomas of BALB/c mice demonstrated that 6.9% of tumors produced 2 or more immunoglobulin classes. Two tumors, SAMM 368 and TEPC 609, have been studied in depth Immunofluorescence studies revealed that single cells from both tumors contain both immunoglobulin products. Evaluation of paraproteins produced by SAMM 368 showed that these products do not share idiotype and that the IgG2b product does not carry the allotype markers of this immunoglobulin class. The paraproteins secreted by TEPC 609 have been shown to share idiotype.

Over 400 plasmacytomas of NZB mice have also been tested for their immunoglobulin products; about 10% of primary ascites contained 2 immunoglobulin products; about 10% of primary ascites contained 2 immunoglobulin classes. One of these tumors, PC2834, produces both IgA and IgG3 and single cells contain by classes as shown by immunofluorescent staining.

The NZB plasmacytomas differ from those of BALB/c mice in that the predominant class of immunoglobulins produced by the tumors is IgG rather than IgA. Paraproteins belonging to each immunoglobulin class defined in BALB/c mice have also been detected among NZB plasmacytoma products. No paraproteins corresponding to the IgE or IgD immunoglobulin class of man have been detected.

Only a few of the NZB plasmacytomas have been found to bind antigens commonly bound by BALB/c paraproteins, e.g., bacterial polysaccharides. Studies are now in progress to determine if any of the paraproteins correspond to "auto antibodies" of NZB mice.

Other studies evaluated the ontogeny of factors influencing the magnitude of the antibody-forming cell (PFC) response to immunization with Type III pneumococcal polysaccharide (SSS-III). It was shown that B cells capable of responding to SSS-III were present in mice 2 weeks of age but that mice did not reach mature (8-12 week) levels of PFC production until 4 weeks of age. Suppressor T cell activity was found to be fully mature in mice 2 weeks of age whereas amplifier T cell activity gradually increased from 2 to 8 weeks of age. These studies indicate that suppressive factors are predominant in young mice and may be of major importance in regulating the immune response to "self" and other antigens to which animals are exposed early in life.

Publications:

Morse, H. C. III, Pumphrey, J. G., Potter, M. and Asofsky, R.: Murine plasma cells secreting more than one class of immunoglobulin heavy-chain. J.Immunol., in press.

Baker, P. J., Morse, H. C. III, Cross, S. S., Stashak, P. W. and Prescott, B.: Maturation of regulatory factors influencing the magnitude of the antibody response to Type III pneumococcal polysaccharide. J.Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00136-04 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Functional Activities of Subpopulations of Thymus-Derived Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Tigelaar	Senior Investigator	LMI/NIAID
OTHER:	R. Asofsky	Laboratory Chief	LMI/NIAID
	C. B. Evans	Biological Laboratory Tech.	LMI/NIAID
	D. Mosier	Senior Investigator	LI/NIAID
	G. Bonnard	Senior Investigator	LID/NCI
	S. Staal	Expert Consultant	LID/NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14/12	10/12	4/12

SUMMARY OF WORK (200 words or less - underline keywords)

Reproducible IgM anti-SIII plaque-forming cell (PFC) responses have been obtained from sublethally irradiated recipients of normal mouse spleen cells. Low-dose tolerance can be induced in normal cells after adoptive transfer, but pretreatment of donors with low doses of SIII 72 hours before transfer does not lead to reduced SIII responses by these cells nor do such cells suppress the response of normal cells. Studies are underway to further explore conditions in which it will be possible to analyze the nature of the cell(s) and/or factor(s) responsible for the regulation of this immune response in the intact animal.

Additional studies are planned to determine whether the immunosuppression previously observed with preparations of minute virus of mice (MVM) in MLR and CML reactions is directed against all cells regardless of the functional activity expressed or rather is directed against specific subpopulations of T or B cells. Assays in which this viral immunosuppression is being studied include T anc B mitogen responses, antigen specific proliferative responses (to soluble antigens, H-2 antigens, and M-locus antigens) and primary in vitro PFC responses to both T-dependent and T-independent antigens.

Project Description:

Project Subtitle I: Regulation of the *in vitro* antibody response to pneumococcal polysaccharide.

The purpose of this study continues to be the elucidation of the mechanisms by which the antibody response to Type III pneumococcal polysaccharide (SIII) is controlled. A substantial amount of circumstantial evidence has implicated two distinct types of regulatory thymus-derived (T) cells, termed amplifiers and suppressors, as playing central roles in this control (see: Z01-AI-00142-03-LMI, 1974-1975). The need for a suitable model, either an *in vitro* response or an *in vivo* adoptive transfer system, in order to more precisely define the nature and mechanism(s) of action of these regulatory cells, has prompted these studies. The *in vitro* antibody response to SIII (see: Z01-AI-00136-03-LMI, 1974-1975) has been rather difficult to pursue because of (a) the recent variability in the magnitude of the plaque-forming cell (PFC) response and (b) the sensitivity of this response to extremely small amounts of antigen (i.e., approximately 10^{-6} µg/culture is optimal amount) and hence also antibody: as a result current efforts in this area have been directed at developing an *in vivo* adoptive transfer model.

The major findings of this study to date have been: (1) Transfer of primary anti-SIII PFC responses in sublethally irradiated (300R) BALB/c recipients with normal BALB/c spleen cells. The optimal dose of SIII (0.5 µg/mouse) and kinetics (peak PFC response day 4-5) are similar to those found in primary responses in intact BALB/c mice. The response is a log-linear function of the number of cells transfered. Better responses are generated when adoptive recipients are immunized 48 hours after cell transfer rather than on the same day as cell transfer.

(2) Induction of low-dose tolerance in irradiated recipients of normal spleen cells: 300R mice given normal spleen cells and then primed with a low dose (.005 µg) of SIII 72 hours before challenge with the optimally immunogenic dose of SIII make significantly fewer PFCs than unprimed animals. The tolerance-inducing capacity is in the adoptively transferred population and not in the cells of the 300R host.

(3) Failure of *in vivo* administered heterologous anti-thymocyte serum (ATS) to enhance the adoptive primary response. Dose-dependent reduction in the response to SIII was seen whether adoptively transferred cells were exposed to ATS in the donor animal for periods from 1-48 hours prior to transfer or in the recipient at the time of immunization.

(4) Failure of cells from donors primed 72 hours previously with 0.005µg SIII either to give a reduced response upon transfer into and subsequent immunization of the adoptive recipient or to suppress the response of simultaneously transferred normal cells. However, preliminary experiments suggest that cells obtained from donors low-dose primed 7-12 days before are specifically unresponsive to SIII immunization in adoptive hosts.

These results suggest that the methods routinely used to demonstrate amplifier or suppressed responses in the intact animal either must be substantially modified in the adoptive transfer model or do not exert the same effects on cells removed from their usual environment. Future studies will be designed in order to distinguish between these alternatives. The antibody response to SIII was one of the first systems in which a crucial role for regulatory T cells was suggested - in light of the current attempts to develop effective vaccines against various pneumococcal infections, it is more important than ever to adequately delineate the nature and mechanism(s) of action of cells which could either markedly enhance or depress the immune response both to a vaccine and to subsequent challenge with living organisms.

Project Subtitle II: In vitro activities of subpopulations of T cells.

This project has involved two major areas of study: (1) definition of the subclasses of T cells involved in alloantigen recognition and the ontogeny of these functional activities and (2) definition of the subclass(es) of cells which is the target for the immunosuppressive activity of the parvovirus, minute virus of mice (MVM).

The first study has utilized two of the commonly employed in vitro correlates of cellular immunity, the mixed lymphocyte reaction (MLR) and the generation of cell-mediated lympholysis (CML) to characterize the activity of thymocytes from BALB/c mice of various ages in their response to irradiated C57Bl/6 target cells. The MLR measures the proliferation induced in lymphocytes by the recognition of major histocompatibility locus determinants: some I region determinants (frequently equated with lymphocyte-defined or LD products) are sufficient to produce a MLR. The assay for CML measures the generation of cytotoxic effector cells and their ability to destroy ^{51}Cr-labeled allogeneic target cells. The effector cells seem to be directed against antigens coded for by the K or D regions of the major histocompatibility complex; such determinants have been termed serologically-defined or SD products. Generation of maximum CML activity seems to depend on cooperation between cells recognizing LD determinants and other cells recognizing SD determinants.

Significant MLR activity was first present at 19 days of gestation but no significant generation of CML activity was detected until one week after birth. The MLR produced by thymocytes from one week old mice was quantitatively as great as that produced by adult cells, but the peak proliferation occurred earlier (days 4-5 of culture) for neonatal cells than for adult cells (days 6-7 of culture). Although the CML activity generated by one week old thymocytes was low, the activity detected also peaked after 5 days of culture. In addition, although the total CML activity was low, the lytic capacity per neonatal cell was similar to that of adult thymocytes, suggesting that either the number of CML precursors was reduced but the activation process was already intact or that the mechanism for expanding the numbers of activated precursors was defective. The level of CML

activity generated increased slowly with age until the mice were 6-8 weeks old (with considerable inter-experiment and inter-litter variation suggesting some uncontrolled environmental maturational factors). The peak level of CML activity in adult cells occurred on the same day of culture as the peak MLR response. In light of recent compelling evidence that the precursors of CML effector cells represent one phenotypically distinct and stable subclass of T cells and that helper (and LD responsive) T cells represent another, the present data is consistent with a model in which differentiation to full functional maturity of these two subsets of T cells within the thymus procedes at different rates.

The second major area of study has involved the use of purified preparations of the parvovirus, minute virus of mice (MVM) in various in vitro assays for immune reactivity in order to more precisely define the target cell(s) for the previously observed immunosuppressive activity of this virus. Published studies showed that culture supernatants of a subline of the C57Bl/6 lymphoma, EL-4, would markedly suppress the peak MLR and CML responses generated in mixtures of allogeneic mouse spleen cells. This activity was removed from the supernatant by centrifugation at 100,000 X g, UV - light exposure, and most importantly with monospecific anti-MVM antisera. It was of interest to study these supernatants in other in vitro assays. Neither the peak responses to T cell mitogens or B cell mitogens nor the primary in vitro PFC responses to T-dependent (sheep red blood cells, SRBC) and T-independent (trinitrophenylated - Ficoll, TNP-Ficoll) were affected by concentrations of supernatant which ablated MLR and CML activity. Although this data was consistent with the MVM acting selectively on alloantigen reactive T cells, other interpretations were also very possible, including insufficient concentration of virus in the supernatant fluid. Thus, additional studies have been carried out with a sucrose density gradient - purified preparation of MVM.

The principal findings to date include;

(1) The purified MVM preparation is 10^2 - 10^3 times more effective than culture supernatants in suppressing MLR and CML responses of BALB/c spleen cells to irradiated C57Bl/6 stimulator cells.

(2) In spite of this enrichment, the MVM preparation has little if any effect on peak (day 2-3) responses of spleen cells to T and B cell mitogens or the PFC responses of normal spleen cells to either SRBC or TNP-Ficoll.

(3) The MVM preparation also has little effect on the peak (day 3) antigen specific T-dependent proliferative response of BALB/c spleen cells immune to P. berghei yoelii (see: Z01-AI-00141-01-LMI, 1974-1975).

(4) Preliminary experiments have shown that the day 5 antigen-specific proliferative response of immune mouse peritoneal exudate T cells to PPD was significantly but incompletely reduced in the presence of MVM; however, the amount of virus needed to achieve this degree of suppression was 10^2 - 10^3 times more than was necessary to completely inhibit the day 5 MLR response (done by Dr. R. Schwartz, see: Z01-AI-0030-07-LI, 1974-1975).

These results are consistent with the hypothesis that MVM exerts a rather selective immunosuppressive action on T cells responding to alloantigens. Additional studies are planned to investigate whether MVM can suppress (a) the proliferative response of mouse spleen cells to M-locus, LD, or tumor-specific antigens, (b) the proliferative response of normal spleen cells to soluble P. berghei yoelii antigen, and (c) the primary PFC response to SRBC of cultures of B cells reconstituted with limiting numbers of T cells.

Characterization of the target cells for the immunosuppressive activity of MVM is important for at least two reasons. First, MVM may be frequently isolated from various mouse colonies and tumor cell lines, and it is possible that this immunosuppressive activity may have an adverse effect in vivo on the host's immune response to a growing tumor. Secondly, a biologic agent with selective activity for distinct subpopulation of lymphoid cells may ultimately prove to be a useful laboratory reagent for facilitating the dissection of the cellular components of complex immunologic reactions.

Publications:

Gorczynski, R. M., and Tigelaar, R. E.: Cell-mediated immunity to murine tumor allografts. Increase in the activities of activated thymus-derived cells following in vitro incubation. Cell Immunol. 18: 121-143, 1975.

Mosier, D. E., Tigelaar, K. E. and Cohen, P. L.: Ontogeny of in vitro correlates of graft-versus-host reactions. Trans.Proc., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00137-10 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biology of Graft-Versus-Host Reactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. G. Herrod	Research Associate	LMI/NIAID
OTHER:	R. Asofsky	Laboratory Chief	LMI/NIAID
	B. J. Mathieson	Guest Worker	LMI/NIAID
	L. Klassen	Research Associate	A&R/NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12/12	12/12	0/12

SUMMARY OF WORK (200 words or less - underline keywords)

Graft-vs.-host reactions were used to detect phenotypic differences between genotypically identical reciprocal hybrid mice. Cells from either postpartum or virgin female mice of the two parental strains were injected into newborn reciprocal hybrid recipients. With all donors CBL recipients (BALB/c♀ X C57B♂) gave greater spleen indices than did BLC's (C57B1♀ X BALB♂). Post partum BALB/c's that had borne CBL litters appeared to be immunized to antigens unique to CBLs. Post partum BALB's also showed more rapid graft rejection of skin from CBL as compared to BLC. There was no difference in the ability of CBL and BLC cells to stimulate in MLC. These results are interpreted as reflecting a maternal modification of fetal antigens.

Project Description:

This years work has been primarily concerned with using the spleen weight and mortality graft-vs-host assays as tools in studying phenotypic differences in genotypically identical reciprocal hybrid mice.

Reciprocal hybrid mice are derived when the females from two inbred strains are mated with males from the alternate strain. Offspring from such matings are considered to be genotypically identical. The hybrids used in these studies were (C57Bl♂ X BALB/c ♀)F_1=BLC and (BALB/c♀ X C57Bl♂) F_1=CBL.

Previous studies from this laboratory have shown that CBLs give greater spleen indicies than do BLCs when given cells from male mice of the two parental strains. These studies also showed that when donor male cells were of the same strain as the maternal strain of the hybrid recipient they were less lethal in the mortality assay.

The present study examined the ability of virgin and post partum cells from females of the two parental strains to detect phenotypic differences between the two hybrid strains. Post partum and virgin female cells were able to respond equally well to irradiated cells from either hybrid in the mixed lymphocyte culture.

In the spleen weight assay greater spleen indicies were seen in CBLs than in BLCs with all donors. BALB/c females that had borne a CBL litter appeared to have been immunized to antigens present on the fetus. A surprising finding was that this apparent immunization was evident only when CBLs were the recipient. Post partum C57Bl mice did not appear to be immunized to either hybrid.

In the mortality assay, differences between the reciprocal hybrids were seen at only one cell dose. 5×10^6 post partum BALB/c cells gave greater mortality than did an equivalent dose of virgin BALB/c cells in both CBLs and BLCs.

Skin grafts from female CBLs and BLCs onto either post partum or virgin BALB/c's were performed. Post partum recipients rejected CBL grafts faster than did virgin recipients thus again suggesting that they had been sensitized previously. CBL grafts were rejected faster than BLC grafts by post partum BALB/c. Post partum and virgin animals rejected BLC grafts at the same rate.

These studies suggest the following: (1) that there are subpopulations of lymphocytes responsible for the results seen in different assays. (2) There are phenotypic differences between genotypically identical hybrids. These differences are probably expressed early in utero and possibly might by affected by the in utero environment.

Work is currently in progress to study the role of the in utero environment on phenotypic expression.

Publications:

Morse, H. C. III and Asofsky, R.: Effects of antithymocyte serum on lymph node cells participating in the graft-vs-host reaction. Cell.Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00138-02 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Viruses and the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. C. Morse III	Senior Investigator	LMI/NIAID
OTHER:	R. Asofsky	Laboratory Chief	LMI/NIAID
	J. W. Hartley	Senior Investigator	LVD/NIAID
	P. J. Baker	Research Microbiologist	LMI/NIAID
	E. D. Johnson	Staff Fellow	NI/NINCDS
	T. Chused	Senior Investigator	LMI/NIDR
	S. S. Cross*		

COOPERATING UNITS (if any)

*Department of Pathology, Medical College of Virginia, MCV Station, Richmond, Virginia.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6/12	6/12	0/12

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the effects of neonatal infection with mouse thymic virus on the immune system showed that the virus has no effect upon B cells and has strikingly differential effects upon subpopulations of T cells; for example, cells producing direct GVH activity in thymus were depleted following infection with thymic virus whereas cells in thymus producing synergy when combined with lymph node cells were normal.

A rabbit antiserum specific for antigens of xenotropic murine leukemia virus has been prepared and is being used in the presence of virus-related cell surface antigens in the lymphoid compartments of different strains of mice. In addition, the genetics of factors regulating the expression of intact virus is being evaluated.

Other studies investigated the immune mechanisms responsible for production of neurologic disease in mice infected with lymphocytic choriomeningitis virus (LCM).

PHS-6040
(12-75)

Project Description:

The purpose of these studies is to evaluate the interaction of viruses and the immune system, both in terms of alterations produced by viruses in cell populations participating in the immune response as well as the immune response to viral antigens.

A murine herpes virus, mouse thymic virus, has been shown to produce necrosis of the thymus at 7-10 days of age when given to newborn mice, followed by gradual recovery of normal thymic structure. Spleen, thymus and lymph node cells from infected BALB/c mice were tested for their capacity to produce a graft-vs-host (GVH) reaction in neonatal F_1 recipients. A major deficiency in GVH-reactivity of thymocytes and spleen cells was demonstrated in mice 4-8 weeks after infection. Lymph node GVH reactivity was affected little if at all suggesting that the virus may selectively affect subpopulations of T cells. This suggestion was supported by studies of the development of suppressor and amplifier T cell populations which modify the response of B cells to immunization with Type III pneumococcal polysaccharide (S3). Infected and normal mice 2-8 weeks of age were immunized with optimally immunogeneic doses of S3 and tested for enhancement of the plaque-forming cell (PFC) response by treatment with antilymphocyte serum as an indirect measure of amplifier T cell function; no major differences between thymic virus-infected and normal mice could be detected at all ages tested. Suppressor T cell function was assessed indirectly by testing the capacity of low-dose S3 priming to reduce the PFC response to an optimally immunogenic dose of S3; again, no differences between infected and normal mice of various ages could be detected suggesting that both suppressor and amplifier T cell functions were relatively spared. These studies give further indications of heterogeneity among T cell populations and suggest that they may be selectively altered by a T-cell-tropic herpes virus.

In other studies, rabbits immunized with rabbit cells containing xenotropic murine leukemia virus (X-MuLV) produced antibodies which neutralize X-MuLV but not ecotropic MuLV or laboratory strains of MuLV. The antiserum also recognizes cell surface antigens of cells infected with X-MuLV but not ecotropic MuLV. Work is now in progress to determine the genetic mechanisms controlling expression of cell surface antigen as some strains of mice (e.g., NZB) express large amounts of antigen whereas other strains (e.g., NSF) produce little if any.

Studies of the pathogenesis of LCM-induced neurologic disease in mice were carried out to determine the role played by antibody and T cells. Mice were treated from birth with antibody to IgM immunoglobulin. This treatment has been shown to abrogate the capacity of mice to respond to immunization with antibody production but T cell functions are not altered. Control and treated mice were inoculated intracerebrally with LCM; both groups of mice died with encephalitis with similar histopathology evident in the brains of infected mice of both groups. These studies demonstrate that antibody plays no role in the development of LCM-induced encephalitis.

Publications:

Morse, H. C. III, Cross, S. S. and Baker, P. J.: Neonatal infection with mouse thymic virus: Effects on cells regulating the antibody response to Type III pneumococcal polysaccharide. J.Immunol., in press.

Cross, S. S., Morse, H. C. III and Asofsky, R.: Neonatal infection with mouse thymic virus: Differential effects on T cells mediating the graft-versus-host reaction. J.Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00139-11 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Immunologic Response of Animals to Trypanosomal Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Finerty	Research Microbiologist	LMI/NIAID
OTHER:	R. L. McKelvin*	Student-Laboratory Worker	LMI/NIAID

COOPERATING UNITS (if any)

*Howard University, Washington, D. C.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/12	4/12	0/12

SUMMARY OF WORK (200 words or less - underline keywords)

Mice immunized with Trypanosoma rhodesiense antigens demonstrated delayed hypersensitivity/or protection. Delayed hypersensitivity could be adoptively transferred by spleen cells from immunized animals. Complete protection to challenge with live organisms occurred within 1 week after immunization.

Project Description:

The purpose of this study was to assess the role of cell-mediated immunity (CMI) in host protection to lethal species of Trypanosoma, causative agents of African Sleeping Sickness. CMI was assayed by a delayed footpad swelling technique, an indication of delayed hypersensitivity (DH). DH, a T-cell function, was measured by the injection of antigen equivalent to 10^8 trypanosomes, into a rear footpad, and the degree of swelling measured 24 hrs. later. Mice immunized with trypanosomal antigens, demonstrated a peak DH response on Day 4. However, mice challenged with Trypanosoma rhodesiense on day 4 were not protected. Complete protection was not demonstrable until 8 days after immunization. Since DH was observed in non-protected and protected animals, this suggests that different groups of antigens are involved in the induction of hypersensitivity vs. those capable of stimulating a protective immune response. Attempts at enhancing T-cell activity by the use of Cyclophosphamide (Cy) were unsuccessful, suggesting that protecting against T. rhodesiense may be a B-cell function. Adoptive transfer of spleen cells into nude mice produced a delayed response to T. rhodesiense antigens. The significance of these studies is that DH can be induced to T. rhodesiense antigens without the use of adjuvants, and that protection can also be induced, without adjuvants, with various trypanosomal antigens. Future studies will assay the type of antigens that induce protection, and the type of cell function mediating this activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00140-11 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunology of Malaria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Finerty	Research Microbiologist	LMI/NIAID
OTHER:	R. Rank*		
	W. P. Weidanz*		

COOPERATING UNITS (if any)

*Department of Immunology and Microbiology, Hahnemann Medical School, Philadelphia, Pennsylvania

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8/12	8/12	0/12

SUMMARY OF WORK (200 words or less - underline keywords)

Delayed hypersensitivity (DH) was produced in mice immunized with Plasmodium berghei antigens. Mice infected with P. berghei revealed little or no DH activity and succumbed to infection. The administration of an alkylating agent, cyclophosphamide increased T cell activity, manifested by heightened DH reactions, and survival of infected animals.

PHS-6040
(12-75)

Project Description:

It is the purpose of these studies to determine the nature of the host immune responses to the rodent malarial parasite Plasmodium berghei, and the role of these responses in host protection. Particular emphasis was focused on cell-mediated immune (CMI) responses, after immunization with antigens prepared from the blood stages of P. berghei or after infection induced by lethal and non-lethal strains of P. berghei. CMI responses were studied in vivo using footpad responses as an indicator or an immediate (Arthus) reaction, or delayed response. The latter is an indication of delayed-hypersensitivity (DH) in the mouse, and has been ascribed to T cell function. Footpad swelling was elicited with soluble or freeze-thawed antigens injected into the plantar surface of a hind footpad. The degree of footpad swelling was measured between 3 and 24 hours after the antigen was injected into the footpad. Mice infected with P. berghei revealed 3 hr. footpad swellings beginning 4 days after infection. These reactions increased in intensity as the parasitemia increased. DH reactions measured at 24 hours in these same mice were minimal, lower than the 3 hour reactions by a factor of 4-6, and all mice succumbed to infection within 8 days. Studies were designed to augment T cell function, by the use of Cyclophosphamide (Cy), an alkylating agent. Subsequently, mice were inoculated intraperitoneally with lethal P. berghei, and footpad swelling measured 2, 4, 6 and 8 days after infection. The 3 hour reactions in the Cy group became evident on day 4, increased in intensity with time after infection, but were lower than non-Cy treated mice. The difference between the Cy and non-Cy groups, in their footpad reactions, was the intense DH reaction observed in the Cy group which peaked 2 days after infection. These Cy treated mice cleared their parasitemia and survived; non-treated mice died. Studies were initiated to determine the effects of Cy upon immunization with malarial antigens. Increased DH reactions were observed in the Cy treated mice. Adoptive cell transfer studies demonstrated that the DH reaction can be transferred by cells. These results are significant for they demonstrate that an enhanced T cell response to lethal P. berghei has a beneficial effect on host survival. Future studies will investigate the effect of Cy on subpopulations of lymphocytes and their role in host protection to the blood stages of malaria.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00141-02 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immune Response to Malaria and Malarial Antigens
(Former Title: T-cell Sensitization in Murine Malaria)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. I. Weinbaum	Research Associate	LMI/NIAID
OTHER:	C. B. Evans	Biological Laboratory Technician	LMI/NIAID
	R. E. Tigelaar	Senior Investigator	LMI/NIAID
	D. Chen	Staff Fellow	LPD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
22/12	14/12	8/12

SUMMARY OF WORK (200 words or less - underline keywords)

Mice injected from birth with goat antiserum to mouse μ chain (μ-suppressed) were utilized to examine the requirement for immunocompetent B-cells in the immune response to 17NL P.b. yoelii malaria (ordinarily non lethal), and to X-irradiated P.b.berghei sporozoites. Previous work showed that T cells were required for resistance of BALB/c mice to 17XNL P.b. yoelii. Present experiments demonstrated increased parasitemia and mortality of μ-suppressed BALB/c mice compared to controls implicating an important role for B cells. In contrast, μ-suppressed $BLCF_1$ mice immunized with sporozoites resisted a challenge infection in the majority of cases. Adult thymectomized, X-irradiated, bone marrow reconstituted, and antithymocyte serum treated $BLCF_1$ mice were not effectively immunized using the same protocol, suggesting an essential role for T cell immunization in sporozoite immunity.

We have studied a variety of parameters during the course of infection of BALB/c mice with 17XNL P.b. yoelii including parasitemia, hematocrit, antimalarial indirect hemagglutinating antibody and in vitro responses of spleen cells to malarial antigens (T dependent) and mitogens. A dynamic series of events was observed, characterized by rapid T cell sensitization and a period of depressed in vitro spleen cell responsiveness during the period of peak parasitemia.

PHS-6040
(12-75)

Project Description:

The purpose of these investigations has been to further delineate the cellular requirements for an effective immune response to malaria infections and malarial antigens. We have previously established the T cell dependence of resistance of adult BALB/c and $BLCF_1$ mice to infection with 17X non-lethal Plasmodium berghei yoelii (17XNL P.b.y.), an infection which is self limited in immunocompetent mice. In the present investigations we have established the B cell dependency of resistance of BALB/c mice to 17XNL P.b.y. by comparing the parasitemia and mortality of mice infected from birth with goat antiserum to mouse μ chain (u-suppressed, B-deficient) to that of controls injected with goat anti-ferritin antiserum. Mice treated with anti-μ from birth have been shown to be unable to make antibody responses to challenging antigens, whereas their T cell responses have been shown to be intact.

To better understand the dynamic events in an infection of BALB/c mice with 17XNL P.b.y. the following parameters were measured at intervals after infection: parasitemia, hematocrit, in vitro proliferative responses to plasmodial antigens, phytohemagglutinin (PHA) and lipopolysaccharide (LPS), indirect hemagglutinating antibody (IHA), and splenic plaque forming cell responses to immunization with sheep erythrocytes (SE). In addition, in collaboration with Dr. D. Chen of the Laboratory of Parasitic Diseases, the cellular requirements for immunization against P.b.berghei sporozoites were determined by comparing the results of attempted immunization of adult thymectomized, X-irradiated, bone-marrow reconstituted, antithymocyte serum treated (ATX-BM-ATS) $BLCF_1$ mice, μ-suppressed $BLCF_1$ mice, $BLCF_1$ mice injected from birth with goat anti-ferritin, and age and sex matched $BLCF_1$ controls.

The major findings of these investigations have been:

(A) μ-suppressed BALB/c mice demonstrated markedly increased parasitemia and mortality (10 of 10) compared to that of controls (2 of 15 succumbed). This confirmed the requirement for immunocompetent B cells for resistance to an ongoing malaria infection that is self-limited in immunocompetent mice, (17XNL P.b.y.).

(B) The following points emerged from a study of the kinetics of various parameters after BALB/c mice were inoculated intraperitoneally with 10^6 RBCs infected with 17XNL P.b.y.:

(1) Parasitemia peaked d.9-11; usually <10%; cleared d.16.

(2) Maximal hematocrit depression coincided with peak parasitemia; as low as 30% packed cell volume.

(3) Initially there was a significant in vitro spleen cell proliferative response to plasmodial antigens (1-2 days after infection). By 3 days after infection, markedly increased background proliferation of unstimulated cultures was observed and obscured responses to plasmodial antigens. By d.7, neither increased background nor responses to antigen were observed. A significant response to antigen was again observed after d.16 and was observed at high levels for at least 7 months thereafter.

(4) In vitro spleen cell mitogen responses (phytohemagglutinin and lipopolysaccharide) were found to be significantly depressed starting 3

days after infection and did not return to normal until 6 weeks (PHA) or 7 months (LPS) after infection.

(5) IHA levels were observed initially 5 days after infection, increased until 18 days after infection and then were stable for at least 7 months.

(6) Anti-sheep cell splenic plaque forming cell responses were depressed severely when mice were immunized 8 days after infection. However, an increased response was observed in mice immunized d. 18.

(C) T-cell deficient (ATX-BM-ATS) $BLCF_1$ mice were not effectively immunized with X-irradiated sporozoites; 0 of 13 mice were protected. In contrast 7 of 11 B-cell deficient mice were protected. All anti-ferritin injected (8) and untreated controls (12) were immune to challenge with viable sporozoites. None of the unimmunized mice (0/30) were resistant to challenge.

(D) The selective nature of the defect in μ-suppressed mice was confirmed by intact splenic in vitro T-cell responses (PHA and mixed lymphocyte culture) in the presence of nearly absent B cell mitogen (LPS, poly I) responses.

Thus we have established the requirement for T cells and B cells for BALB/c mice to resist an infection with RBCs infected with 17XNL P.b.y.. In contrast, immunization with X-irradiated sporozoites appears to be T cell dependent, but not B cell dependent, although B cells may play a minor role. The dynamic processes which occur during the course of a nonlethal malaria infection (17XNL P.b.y.) of BALB/c mice include initial T cell sensitization (in vitro response to malaria antigens), early antibody production (d. 5), and a period of elevated in vitro background proliferation, followed by depressed in vitro spleen cell responses and in vivo splenic anti-SE plaque forming cell responses when the parasitemia is most active. As the parasitemia is cleared, spleen cell responsiveness return.

One mechanism to explain the requirement for T cells and B cells in immunity to an ongoing malaria infection is a requirement for T dependent antibody production. However, data from the literature showing that transferred immune serum effected only partial protection suggests another role for T cells. The demonstration of activation of the reticuloendothelial system in rodent malaria and the effect of BCG and Corynebacterium parvum has suggested an important role for macrophage activation. Studies are presently underway to examine the protective effects of passive immune serum transfer in conjunction with nonspecific stimuli of macrophage activation on a lethal (17XL P.b.y.) malaria infection.

The immune response to sporozoites is similarly being pursued by an examination of the effects of BCG on sporozoite immunization and challenge.

Other areas of investigation include:

1. A comparison of the various parameters measured in 17XNL P.b.y. infections with those observed in 17XL P.b.y.

2. Investigations into the mechanism of immunosuppression in malaria infections utilizing in vitro primary immune responses to probe the nature of the defect at the cellular level.

3. The effect of malaria induced immunosuppression on the immune response to other infectious agents (lymphocytic choriomeningitis) and oncogenic viruses (Abelson virus).

Publications:

Weinbaum, F. I., Evans, C. B., and Tigelaar, R. E.: An in vitro assay for T-cell immunity to malaria in mice. J.Immunol. 116: 1280-1283, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00142-04 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of Thymus-Derived (T) Suppressor and Amplifier Cell Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. J. Baker	Acting Head, Microbiology and Immunology Section	LMI/NIAID
OTHER:	H. C. Morse III	Senior Investigator	LMI/NIAID
	B. Prescott	Head, Biochemistry Section	LMI/NIAID
	P. W. Stashak	Microbiologist	LMI/NIAID
	D. F. Amsbaugh	Biologist	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive during current year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00143-07 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Genetic Control of the Antibody Response to Type III Pneumococcal Polysaccharide (SSS-III)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. J. Baker	Research Microbiologist	LMI/NIAID
OTHER:	B. Prescott	Microbiologist	LMI/NIAID
	D. F. Amsbaugh	Biologist	LMI/NIAID
	P. W. Stashak	Microbiologist	LMI/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
20/12	8/12	12/12

SUMMARY OF WORK (200 words or less - underline keywords)

An analysis of the strain distribution patterns for recombinant-inbred strains of mice immunized with Type III pneumococcal polysaccharide (SSS-III) showed that (a) at least 3-5 genes influence the capacity of bone marrow-derived precursors of antibody-forming cells (B cells) to make an antibody response, (b) at least 2 genes control the expression of suppressor T cell activity, and (c) at least 3-4 genes govern amplifier T cell function. These autosomal immune response genes act independently and in a complementary manner to produce the effects observed. They are not linked to genes within the major histocompatibility (H-2) or immunoglobulin allotype complex.

PHS-6040
(12-75)

Project Description:

Previous studies conducted in this laboratory have shown that the antibody response of mice to SSS-III is under at least two different forms of genetic control. First, there is a gene linked to the X chromosome that determines the ability to make an IgM antibody response in an almost "all-or-none" manner. Second, there are autosomal genes that influence the magnitude of the antibody response of mice possessing the X-linked gene. Although an analysis of the effects produced by these quantitative genes would shed much light on the mechanisms by which antibody responses are initiated and controlled, this task is made difficult by the fact that three interacting cells, whose activities may be under separate genetic control, participate in the antibody response to SSS-III. They are: bone marrow - derived precursors of antibody-forming cells (B cells); thymus-derived (T) suppressor cells, which limit the extent to which B cells proliferate in response to antigen; and, amplifier T cells, which appear to regulate the rate of antibody synthesis by B cells. The combined activities of all of these cells contribute to the magnitude of the antibody response produced after immunization.

Recombinant-inbred strains of mice, as well as progenitor strains from which they were derived, were evaluated with respect to each of the activities. None of these functional activities was found to be linked to genes within the H-2 or immunoglobulin allotype (IgC_H) complex (see Project No. Z01 AI 00143-06 LMI, 1975). More important, several autosomal genes appear to govern the expression of each characteristic. An analysis of the strain distribution patterns obtained revealed that at least 3-5 genes were involved in regulating B cell function, at least 2 genes controlled the expression of suppressor T cell activity, and at least 3-4 genes governed the activities of amplifier T cells. These genes were found to act both independently and in a complementary manner. Backcrossess between low responding recombinant-inbred strains and low responding progenitor strains yielded progeny which gave high responses to SSS-III. Studies are underway to determine the extent to which these genetic factors influence the immune response to other antigens of biomedical significance, and whether these activities are associated with genetic markers at other known loci.

Publications:

Baker, P. J., Amsbaugh, D. F., Prescott, B., and Stashak, P. W.: Genetic control of the antibody response to Type III pneumococcal polysaccharide in mice. III. Analysis of genes governing the expression of regulatory T cell activity. J.Immunogenetics, in press.

Baker, P. J. and Prescott, B.: The basis for conflicting results obtained in studies on the plaque-forming cell response to Type III pneumococcal polysaccharide. J.Immunol. 116: 891-892, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00144-12 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Regulation of the Antibody Response to Type III Pneumococcal Polysaccharide (SSS-III)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. J. Baker	Research Microbiologist	LMI NIAID
OTHER:	R. B. Markham	Research Associate	LMI NIAID
	B. Prescott	Microbiologist	LMI NIAID
	P. W. Stashak	Microbiologist	LMI NIAID
	D. F. Amsbaugh	Biologist	LMI NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12/12	8/12	4/12

SUMMARY OF WORK (200 words or less - underline keywords)

Treatment with hydrocortisone succinate significantly reduced the magnitude of the plaque-forming cell (PFC) response to the helper T cell dependent form of Type III pneumococcal polysaccharide (SSS-III), but had no effect on the response to SSS-III, with or without treatment with antilymphocyte serum. Also, this drug had no influence upon the induction or expression of suppressor T cell activity. These findings imply that helper T cells are steroid sensitive, whereas those T cells (amplifier and suppressor cells) which regulate the magnitude of the antibody response to SSS-III are steroid resistant.

PHS-6040
(12-75)

Project Description:

It is known that the antibody response to Type III pneumococcal polysaccharide (SSS-III) is controlled by at least two functionally distinct sub-populations of thymus-derived (T) lymphocytes, which apparently differ in their sensitivity to antilymphocyte serum (ALS), an immunosuppresive agent. Consequently, studies were initiated to examine the effects of other sub-populations of T cells involved in antibody responses. SSS-III, a helper T cell independent antigen, was converted to a helper T cell dependent hapten by attachment to horse erythrocytes (HRBC); mice were then immunized with this complex (SSS-III-HRBC) and the effect of treatment with hydrocortisone sodium succinate on both the helper T cell dependent and helper T cell independent antibody responses to SSS-III was assessed.

Treatment with this steroid suppressed the antibody response to SSS-III-HRBC, but had no significant effect on the response to SSS-III, with or without treatment with ALS; this implies that helper T cells required for the response to SSS-III-HRBC are steroid sensitive, whereas those T cells (amplifier and suppressor cells) which regulate the magnitude of the antibody response to SSS-III are steroid resistant. This view was strengthened by the fact that prior treatment with steroid interfered with the development of an augmented antibody response to SSS-III-HRBC in mice previously given carrier alone (HRBC). By contrast, steroid treatment had no effect on both the induction or expression of low-dose paralysis to SSS-III; this is an antigen-specific, T cell dependent form of unresponsiveness in which suppressor T cells play an active role.

The effect of other immunosuppressive agents on the expression of these and other regulatory T cell functions is being investigated. Information derived from such studies will permit a more rational approach to the use of immunosuppressive agents in organ transplantation studies and in the treatment of "auto-immune" diseases. This will be especially true as auto-immune diseases become better defined in terms of defects in particular sub-populations of functionally distinct lymphocytes.

Publications:

Jones, J. M., Amsbaugh, D. F., and Prescott, B.: Kinetics of the antibody response to Type III pneumococcal polysaccharide (SSS-III). I. Use of ^{125}I-labeled SSS-III to study serum antibody levels, as well as the distribution and excretion of antigen after immunization. J.Immunol. 116: 41-51, 1976.

Jones, J. M., Amsbaugh, D. F., and Prescott, B.: Kinetics of the antibody response to Type III pneumococcal polysaccharide. II. Factors influencing the serum antibody levels after immunization with an optimally immunogenic dose of antigen. J.Immunol. 116: 52-64, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00145-09 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. J. Baker	Research Microbiologist	LMI/NIAID
OTHER:	R. B. Markham	Research Associate	LMI/NIAID
	B. Prescott	Microbiologist	LMI/NIAID
	P. W. Stashak	Microbiologist	LMI/NIAID
	D. F. Amsbaugh	Biologist	LMI/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
16/12	8/12	8/12

SUMMARY OF WORK (200 words or less - underline keywords)

Both suppressor and amplifier T cell activity were found to be present in lymphoid tissues other than the spleen. Significant suppression (>90%) could be demonstrated following the transfer of immunologically unresponsive lymphoid cells to normal intact mice immunized with Type III pneumococcal polysaccharide; however, the degree of suppression noted differed from experiment to experiment. The variables involved in these studies are being assessed systematically, so that this experimental approach can be used to obtain information concerning the mechanism by which both types of regulatory T cells are activated during the course of an immune response.

Project Description:

There is much evidence to indicate that suppressor T cells are activated during the course of an immune response to Type III pneumococcal polysaccharide (SSS-III), and that such cells act mainly to limit the extent to which antibody-forming cells proliferate following immunization. However, little is known concerning the mechanism by which these cells are activated. Since the inhibitory effects produced by suppressor T cells are antigen-specific, and since T cells have not been found to recognize and/or respond directly to SSS-III, we proposed that the initiation of an antibody response per se signals the activation of suppressor T cells, and that the idiotypic determinant of cell-associated antibody specific for SSS-III serves as the recognition unit in this regard. If this is the case, then suppressor T cells would function within the framework of a homeostatic control mechanism for regulating immune responses in general; such a mechanism would have important implications concerning the development of lymphoproliferative disorders and auto-immune disease. In order to test this hypothesis, more information must be acquired concerning the distribution of suppressor T cells in various lymphoid tissues; also, methods must be devised which enable one to transfer suppression to normal animals, so that the structural units involved in their activation can be identified.

The results of current studies conducted in our laboratory show that the activities of both suppressor and amplifier T cells are demonstrable in splenectomized mice, which make a very low - but measurable - antibody response to SSS-III; this occurs despite the fact that antibody-producing plaque-forming cells (PFC) can only be found in the spleens of intact mice immunized with an optimally immunogenic dose of SSS-III. Thus, suppressor T cells are not confined exclusively to the spleen. Both spleen cells and thymocytes obtained from mice in a state of low-dose paralysis, a form of unresponsiveness in which suppressor T cells play an active role, gave a significant reduction (>90%) in the magnitude of the PFC response upon transfer to normal mice; however, the degree of suppression obtained (30-90%) varied from experiment to experiment. Clearly, much more information is needed concerning the optimal conditions required for producing such an effect. The results of such studies, which are now in progress, will enable one to select lymphoid cell populations, rich in suppressor T cell activity, for further analysis and characterization.

Publications:

Baker, P. J.: Homeostatic control of antibody responses: A model based on the recognition of cell-associated antibody by regulatory T cells. Transplant.Rev. 26: 1-20, 1975.

Jones, J. M., Amsbaugh, D. F., Stashak, P. W., Prescott, B., Baker, P. J. and Alling, D. W.: Kinetics of the antibody response to Type III pneumococcal polysaccharide. III. Evidence that suppressor cells function by inhibition of the recruitment and proliferation of antibody-producing cells. J.Immunol. 116: 647-656, 1976.

Morse, H. C. III, Prescott, B., Cross, S. S., Stashak, P. W. and Baker, P. J.: Regulation of the antibody response to Type III pneumococcal polysaccharide. V. Ontogeny of factors influencing the magnitude of the plaque-forming cell response. J.Immunol. 116: 279-287, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00146-03 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chemical, Biochemical and Immunological Studies on Biologically Active Fractions Isolated from Various Plant and Bacterial Sources

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Prescott	Head, Biochemistry Section	LMI/NIAID
	P. J. Baker	Acting Head, Microbiology and Immunology Section	LMI/NIAID
OTHER:	G. Caldes	Research Chemist	LMI/NIAID
	M. Gottlieb	Staff Fellow	LPD/NIAID
	C. Thomas II*	Research Plant Pathologist	
	C. Thomas III**	Student	

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
24/12	12/12	12/12

SUMMARY OF WORK (200 words or less - underline keywords)

Further studies on the isolation, purification and characterization by chemical, biochemical and immunological procedures of biologically active polysaccharides from a number of pneumococcal types and from certain plant materials were carried out. Serological cross-reactions of certain plant polysaccharides was observed in the precipitin tests with rabbit and horse antisera to pneumococcus Types I and III. A polysaccharide isolated from Carthemus tintorius (safflower) also cross reacted with Streptococcus A and D and with Mycoplasma pneumoniae antisera in the complement-fixation test. The properties and reactions of the cross-reactive polysaccharides may be of considerable interst in the possible role of cross-reactive antigens in immunological reactions.

Project Description:

I. Lipids:

A. The comparative study of lipids from plant and bacterial sources has been of interest of this laboratory for several years. Recent studies on lipid chemistry have provided us with an insight into the lipid composition and structural similarities present in plant and bacterial materials studied. In previous reports, we have shown that glycolipids isolated from several plants possessed cross-reactivity with Mycoplasma pneumoniae and Streptococci groups A and D. A continuation of these studies on cross-reactive or shared determinants with these and other organisms (pneumococci) is being studied with an additional group of twenty plants. The methods used for the isolation and purification of the serologically reactive plant glycolipids have been described in previous reports (64-C/1969-1970). Glycolipids of daucus carota (wild carrot), carthamus tinctorius (safflower), poterium spinosum (thorney burnet), lawsonia inermis (henna), salvia verbenica (sage), and artemisa dracunculus (tarregon) cross-reacted in serological tests (complement-fixation) with rabbit and human antiserum to M. pneumoniae. These findings show that certain plants and M. pneumoniae share similar lipid antigens.

The lipid extracts from the various plants are also being tested by the Cancer Institute for potential antitumor activity.

B. Collaborative studies with Dr. Gottlieb of the Laboratory of Parasitic Diseases on the lipids of Crithidia fascicolata were carried out. Relatively few studies have been made on the detailed lipid composition of certain parasites and the role they play in the serology of the organism. The study of lipids from the above mentioned parasite have shown that the organism possesses several phospholipids. Purification of crude chloroform-methanol (2:1 v/v) extracts of the organism on columns of Sephadex G-25 followed by chromatography on columns of DEAE-cellulose and silicic acid gave fractions eluted with chloroform, acetone, and chloroform-methanol (1:1 v/v) rich in phospholipids only. The total yield of lipid was 2.7%. The results of thin-layer chromatography and analytical studies have indicated that there are no detectable glycolipids present in the chloroform-methanol (CM 2:1 v/v) lipid extracts of this parasite. The lipids present in C. fascicolata may fulfill a structural role and may be of interest in the possible role they play in pathological conditions.

II. Chemotherapy:

Previous work in this laboratory has shown that certian sulfones may be potential chemotherapeutic agents as antibacterials and antimalarials. The drug development branch of NCI has shown an interest in certain hydrazones and sulfone derivatives. One of the hydrazones - prepared in our laboratory - the hydrazone of 2-hydrazinopyridine and 2,4-dihydroxybenzaldehyde has shown confirmed activity in the 9 K B test system. This compound is now being evaluated in more detail.

Derivatives of 2-chloro-4-(p-nitrophenylsulfonyl) acetanilide are of interest to the NCI program for testing as potential antitumor agents since it was shown that amine derivatives of these nitro compounds possessed antimalarial activity against Plasmodium berghei in experimentally infected mice. The present study extends the series to include related compounds and the effect of molecular structure on the biological properties of the new derivatives. Accordingly, a series of about 25 amine condensation products of the nitrosulfone is being prepared to provide samples for antitumor studies. The derivatives being prepared will contain a variety of functional groups including halogen, carboxyl, nitro, and hydroxyl. The compounds are easily prepared by condensing various amines with the 2-chloro-4'-(p-nitrophenylsulfonyl) acetanilide in absolute ethanol. Final crystallized products will be analyzed for carbon, hydrogen, nitrogen and tested for melting points. The original series of nitrosulfones studied as potential antimalarial agents showed no toxic side reactions which might be attributed to the test substances in the animals receiving 2g/mg of compound. Because of high tolerance, this type of compound may be useful as potential antitumor agents.

III. Polysaccharides:

A. In the course of the study of the potential antitumor activity of certain plant extracts (organic solvent soluble and water soluble) it seemed desirable to investigate the possible antigenicity of the polysaccharides from the plants being studied in our laboratory. Methods successfully employed in pneumococcal studies are being utilized and further developed in plant studies where possible. However, a major effort in applying newer knowledge and techniques from not only recent developments in deriving cellular constituents by mild physical and chemical methods but also by new chemical procedures evolved in our laboratory. The investigations indicated that the polysaccharides from several plant materials cross-reacted with Type III antipneumococcal serum in the precipitin test. Further evidence for this cross-reaction is the immunological properties of one of the plant polysaccharides (carthamus tinctorius) in yivo in mice. The polysaccharides isolated from safflower, shallot, globe daisy and dictamon showed cross-reactivity with Type III pneumococcal antiserum. The C. tinctorius polysaccharide also cross-reacts with M. pneumoniae and streptococcus group A antisera in the complement-fixation test. Extensive work was carried out with the safflower polysaccharide because of the availability of the plant material.

B. Extraction of the polysaccharides from plants was accomplished by H_2O extraction of ground material with subsequent precipitation and reprecipitation from 2% NaAc. Additional purification of the polysaccharides were carried out with chloroform-n-butanol-acetic acid with stirring for 8 hours at room temperature. The mixture was centrifuged and the water layer preciptated with 2 volumns of cold, 95% ethanol. Redissolved in 2% NaAc; filtered through an EK Seitz filter pad, dialyzed and lyophilized. The H_2O extracted residue was taken up in water and diluted with one vol. of N-NaOH with precipitations of the NaOH soluble with subsequent 95% ethanol precipitation followed by reprecipitation from 7.5% trichloracetic

acid. Final preparation with this procedure was similar to the above water procedure. The same procedures have been used for the isolation of polysaccharides from a number of additional plants. All polysaccharide fractions isolated from the plants studied will be tested by NCI for potential antitumor activity.

In collaborations with Dr. C. A. Thomas III, plant pathologist of U. S. Department of Agriculture Experimental Station in Beltsville, Md., extensive studies were performed with Carthemus tinctorius (safflower) leaves, stem, seeds and flowers grown in a greenhouse at various age levels 2 and 4 months. Since the polysaccharide isolated from this plant showed cross-reactions with Type III pneumococcal antiserum, safflower plants are being field grown for further studies.

C. Analytical studies of the purified polysaccharide from Carthemus tinctorus showed the following results: The preparations contained only detectable amounts of nitrogen and phosphorus, and about 3% ash. A solution of the polysaccharide in water was levorotatory $[\alpha]_D^{25} = -31^\circ$.

Carbohydrate analysis: The carbohydrate components of the purified C. tinctorius polysaccharide with high cross-reactivity in the precipitin test with Type III pneumococcal antiserum were analyzed quantitatively by descending paper chromatography. A sample was hydrolyzed with 2N-H_2SO_4 for 2 hours in a sealed tube at 110°C and the hydrolysate submitted to paper-chromatography. This cross-reactive plant polysaccharide was composed of galactose, glucose, arabinose, xylose, mannose and uronic acid. Galactose and glucose are the main carbohydrate determinants. The component sugars on the paper were detected with aniline malonate. Studies to determine and confirm and complex carbohydrate structure and components of the C. tintorius polysaccharide were also carried out with enzyme hydrolysis. Thus, a number of the structural, physical and chemical characteristics of this polysaccharide resemble the Type III pneumococcal polysaccharide.

Polysaccharides were isolated from Mycoplasma pneumoniae and from Streptococcus pyogenes group A and purified by procedures used for pneumococcal polysaccharides. The M. pneumoniae polysaccharide was prepared for collaborative studies with Dr. Helmut Brunner of the Institut fur Medizinische Mikrobiologie der Universitat, 63 Giessen Friedrechstr 16, West Germany and for Dr. Peter Allen, Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. Attempts to use the material as an immunizing agent will be carried out in experimental studies. The streptococcal polysaccharide will be used in experiments coating RBC.

Additional batches of Types 8 and 14 pneumococcal polysaccharides were prepared for further studies on their immunogenicity and Type 3 for large scale immunogenetic studies.

Pneumococcal C-substance prepared from Types I, II and III pneumococci by acid extraction of these organisms were purified by column chromatography

on DEAE-cellulose. The method used in isolating and purifying this material was a modification of the procedure of Brundish and Baddiley. The final products were distributed to Dr. Potter of NCI and Dr. Segal of LI for their studies on the effect of the material on cells of the immune system.

Publications:

Prescott, B.: Thiosemicarbozones and hydrazones of α.-methylchalkone as potential chemotherapeutic agents. Inter.J.Clin.Pharmacol.Biopharm. 11: 332-335, 1975.

Caldes, G. and Prescott, B.: A simple method for the detection of trehalose by spot elution paper chromatography. J.Chromatography 111: 466-469, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00153-01 LMI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

In Vitro Response of Human Peripheral Lymphocytes to Infectious Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	F. I. Weinbaum	Research Associate	LMI/NIAID
OTHER:	R. E. Tigelaar	Senior Investigator	LMI/NIAID
	D. Wyler	Research Associate	LPD/NIAID
	J. Weintraub	Research Associate	LPD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/12	4/12	0/12

SUMMARY OF WORK (200 words or less - underline keywords)

Human peripheral blood lymphocytes were fractionated into either thymic dependent (T) cells or thymic independent (B) cells. Unfractionated cells and T cells were shown to be stimulated by phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM). B cells did not respond to any of these mitogens unless a few T cells were added to the culture. Unfractionated and T cells were capable of proliferating in the presence of Plasmodium falciparum maleria antigens B cells did not proliferate even when T cells were added to the culture.

PHS-6040
(12-75)

Project Description:

The purpose of these studies was to determine what cell type was proliferating most actively in vitro in the presence of P. falciparum antigen. A technique was developed to fractionate peripheral blood lymphocytes (PBL) into thymus derived (T) cells and non-thymic dependent (B) cells. Briefly, PBL's were passed over an anti-human Fab column, B cells were retained on the column because of their surface immunoglobulin while T cells passed through the column. Recovery of both populations of lymphocytes showed them to be highly enriched for either B cells or T cells. A further purification step involved rosetting each of the enriched fractions with sheep erythrocytes. In the case of the T cells the rosetted population of cells was recovered. Those cells in the B cell fraction that did not rosette were considered to be highly purified for B cells. In vitro stimulation of these fractions of cells showed very good stimulation of T cells with PHA, Con A and Pokeweed mitogens. The B cell fraction failed to respond to any of these mitogens. Adding a small number of T cells to the B cell fraction resulted in much higher incorporation of tritiated (^{3}H) thymidine than could be accounted for by the small number of T cells alone.

In the presence of P. falciparum antigen both unfractionated and T cells proliferated very well. B cells showed no increase in incorporation of ^{3}H thymidine above background. Adding T cells to the B cell fraction resulted in no greater incorporation of ^{3}H thymidine than expected from the T cells alone.

Current experiments are underway to study the responce of immune cells. Studies are also being conducted on the response of human PBL to leishmania antigens in vitro.

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Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - July 1, 1975 through June 30, 1976

INTRODUCTION

The past year may be indicative of a new pattern for short term professional personnel in LPD; namely, more Staff and Visiting Fellows and fewer Research Associates than in past years. Drs. Martin, Kassim, and Gusmão are new Visiting Fellows and Dr. McAuliffe a new Staff Fellow. We had no new Research Associates in 1975 and of the three Associates finishing their two year terms, Dr. Weintraub will stay on for another year, while Drs. Mason and Weller will leave. However, two former Associates, Drs. Nash and Wyler, will be rejoining us as permanent staff after having taken special training in clinical infectious diseases and polysaccharide chemistry in Boston.

An important and very productive working assignment by Dr. Kuberski in Fiji on dengue hemorrhagic fever during early 1975 was inadvertently omitted from last year's report. During the past year Dr. Tesh was the guest of the Iranian government for several months studying sandfly fever, a Phlebotomus transmitted virus infection. While attending a meeting in Brazil, Dr. Neva was able to collect infected triatomine bugs for laboratory studies in an area previously sampled. Dr. Neva also visited India to explore the possibility of collaborative work by LPD with Indian scientists on immunological aspects of filariasis.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - July 1, 1975 through June 30, 1976

RESEARCH ACCOMPLISHMENTS

MORE ON THE DUFFY RECEPTOR ON RED CELLS FOR MALARIA INVASION

Further proof was developed for the critical role of the Duffy blood group antigenic determinant for invasion of the red cell by Plasmodium vivax. This has been postulated last year by the finding that Duffy negative erythrocytes were resistant to invasion by the monkey malarial parasite, P. knowlesi, and by the known association of relative frequencies of resistance to P. vivax malaria in African and American blacks. Determination of the Duffy phenotypes of eleven black individuals previously exposed to Vivax infected mosquitoes showed that the five Duffy negative blacks were the only ones resistant to infection; the six blacks who became infected were Duffy positive. Further support for the role of the Duffy receptor came from the finding that Duffy negative red cells from two non-blacks, a rare mutation, were refractory to invasion in vitro by P. knowlesi. However, some aspects of the relationship between blood group determinants and their role as receptors for the malaria parasite are still not clear. Such loci probably do not serve as direct receptors for other species of malaria, such as P. falciparum. Also, the story on P. knowlesi invasion is not yet complete since Duffy a and b determinants do not appear to be the only receptors in some circumstances, such as with red cells of New World monkeys which can be invaded and are Duffy negative. (Miller, Mason, Dvorak, Shiroishi and McGinniss [of the Blood Bank, CC]).

MALARIAL GAMETOCYTE PHYSIOLOGY AND IMMUNITY

Gamete fertilization: While the work described above has focused upon the red cell or asexual stages of the malarial life cycle the gametocytes and their development in the mosquito involving the sexual stage of the cycle have also received attention. Critical factors have been identified that are necessary for successful development of the male and female gametocytes and fertilization of the latter within the mosquito after taking a blood meal. Bicarbonate ion must be present and a very narrow pH range of about 7.9 to 8.1 is optimum for the early part of the process called exflagellation. Definition of these conditions now makes it possible to test different conditions that might influence the process. (Carter and Nijhout).

Immunization vs gametocytes - a new approach: With the development of new techniques that permit controlled manipulation of gametocytes, such as feeding of mosquitoes through membranes and the information as noted above, it has become possible to consider immunologic attack vs the gametocyte. Using an avian malaria model, P. gallinaceum, it was possible to show that immunization with infected blood cells impaired the ability of the animal to transmit the parasite to mosquitoes even though there was no significant effect upon the parasitemia. This was shown to be due to a serum factor generated in the immunized animal that inhibited normal development of the gametocyte. (Gwadz). This approach has been pursued further with P. gallinaceum by isolating and

purifying gametocytes as antigen for immunization and trying to learn more of the mechanism which affects gametocytes. (Carter, Nijhout, Martin, Chen and Green of LI). Thus, gametocyte immunization could be another approach to control of malaria transmission.

Mechanism of immunity to sporozoites: The approach of immunization to malaria with sporozoites was developed fairly recently by Nussenzweig and coworkers, but the immunologic mechanism responsible has never been delineated. Using P. berghei in mice subjected to various combinations of T-cell deprivation or B-cell inhibition it was shown that successful immunization with sporozoites was T-cell dependent and was not necessarily associated with the development of circum-sporozoite precipitating antibodies. In contrast, the majority of animals rendered B-cell deficient were protected after sporozoite immunization. Thus, B-cells may aid in the immune response to sporozoites but there is an absolute requirement for T-cells. (Chen and Weinbaum of LMI).

MORE BASIC INFORMATION NEEDED ON ANOPHELINE PHYSIOLOGY

Since most basic studies of mosquito physiology have been done with culicines, especially Aedes aegypti, a great backlog of fundamental information is needed on the anophelines which are vectors of malaria. It is from such data on feeding and reproduction, endocrine function, etc., that come the ideas for manipulating critical processes that might be exploited for purposes of vector control or interruption of parasite cycle. A modest investigation into this area has been made. Although endocrine regulation of ovarian development in Anopheles was similar to that in A. aegypti several phenomena unique to Anopheles were identified, such as the structure of nurse cell chromosomes and formation of the raft chorion. The passage of different food sources, such as blood or sugar solutions, to either stomach or diverticulum has thus far been found to be similar in Anopheles and A. aegypti. Erection antennal fibrillae or antennal hairs in the male Anopheline mosquito was found to be a useful index of mating activity. The time of occurrence was found to be under photo-periodic influence and mediated by catecholamine or catecholamine-like neurotransmitters. Antennal hair erection may be stimulated by certain other substances as well. (F. Nijhout and M. Nijhout).

PARASITE BIOCHEMISTRY AND ENERGY METABOLISM

In amebae: Productive collaboration with Dr. Diamond's section (see AI-00094-16) which provides bacteria-free E. histolytica has focused mainly upon the role of iron-sulfur proteins in the amebal respiratory chain. Chemical analyses of a partially purified amebal diaphorase (catalyst for oxidation of NAD(P)H) disclosed the presence of non-heme iron and an acid-labile sulfide in a 1:1 molar ratio. This diaphorase has been further characterized by electron paramagnetic reasonance spectroscopy (EPR). The EPR spectra of diaphorase preparations isolated from amebae grown in the presence of ^{57}Fe showed a broadened but otherwise identical signal and analyses by mass spectroscopy confirmed that isotopic substitution had occurred, providing unequivocal evidence that the EPR signal arises from an iron-sulfur center. (Weinbach, Diamond, Harlow, Claggett and Kon of NIAMDD). Continued studies on the newly discovered enzyme pyruvate oxidase (CoA acetylating), now officially recognized by the International Union of Biochemistry, showed it to contain non-heme iron and flavin after purification by hydroxyapatite and aminoethyl cellulose chromatography. Another diaphorase, associated with

the oxidase, was found and it contains two non-heme iron centers; one gives the characteristic EPR signal and the other does not. (Takeuchi and Weinbach). In view of the important role of iron-sulfur proteins in the metabolism of E. histolytica the nutritional requirements of iron and sulfur are being examined. For example, the requirement for relatively large amounts of sulfur-containing compounds in axenic culture media for amebae has been known. A new sensitive technique for analysis of heavy metals, called X-ray energy spectrometry, has proved useful and has shown that different media contain differing amounts of iron and that the amebae concentrate iron from the culture media. (Harlow, Diamond and Weinbach).

Malaria parasites: Comparative studies have been initiated with malaria parasites that contain mitochondria (P. inui) and a species without mitochondria (P. knowlesi). Optical spectrophotometric analyses have disclosed different heme pigments in the two species. The parasites of P. knowlesi appear by EPR analysis to contain an iron-sulfur compound similar to rubredoxin which is totally lacking in P. inui. (Harlow and Miller).

Trypanosomes: Although these protozoa lack glycogen or other energy reserve type carbohydrates, they contain considerable polysaccharide. These polysaccharides which have been extracted by chemical fractionation procedures are being examined for biochemical as well as immunological properties. Two main factions have been recovered from an insect parasite, C. fasciculata: a 14,000 molecular weight mannan and another fraction containing arabinose and galactose. More than 80 percent of the polysaccharide is located in the particulate fraction of the cell homogenate. In contrast, the crude carbohydrate from the human parasite, T. cruzi, is composed primarily of acidic polysaccharides which resolve into at least 3 components on gel electrophoresis. Hydrolysis of the crude fraction yields galactose and mannose as the major neutral sugars. Antibodies have been produced in rabbits against polysaccharide fractions from both species and will be used to localize these components in the cell (Gottlieb).

Energy metabolism in bacterial and mammalian systems: Non-heme iron was also shown to function in the respiratory chain of Staphylococcus areus. (Weinbach and Theodore of LSD). Oxidative phosphorylation in isolated mitochondria was studied from livers of rats in which porphyria had been induced chemically. Rates of NADH oxidation were increased in hepatic mitochondria from experimental animals. (Weinbach and Tschudy, NCI).

HELMINTHS AND HELMINTHIC DISEASES

Schistosomiasis pathology: Results of the computer analysis have now become available of the large autopsy study in Egypt involving 400 cases in which intensity of infection was correlated with pathologic lesions. Although infection with both species of schistosomes, S. mansoni and S. hematobium, was present, severe liver disease attributable to schistosomiasis correlated closely with heavy S. mansoni infections. Pathologic changes as well as intensity of infection were similar to that previously found in Brazil. A major difference in S. mansoni infections in the two countries was the frequent presence of schistosomal polyposis of the colon in Egypt and its absence in Brazil. Patients with obstructive uropathy showed the heaviest S. hematobium infections but

there was no correlation between the degree of obstructive uropathy and intensity of infection (Cheever, Kamel, Elwi, Mosimann and Danner).

Schistosomiasis immunology: A good start has been made in understanding more of the human immunologic response to schistosome infection in collaboration with workers in Puerto Rico (see also LCI Report AI-00047). Cell mediated immune responses to parasite antigens were enhanced in patients with acute infections while chronic cases had greatly diminished reactivity. It was of interest that several of the acute cases, generally thought of as representing individuals with a single heavy exposure to the parasite, had surprisingly low egg counts. This raises a question as to what are the factors in pathogenesis of the striking and often severe clinical manifestations of acute schistosomiasis. (Ottesen, Cheever and Neva).

Snail vectors of schistosomiasis: Further observations upon the two substrains of S. mansoni from St. Lucia derived from what was considered a single parasite strain have disclosed striking differences in their infectivity for various genetic stocks of snails. Also, a recently isolated strain of S. mansoni from Puerto Rico shows a much more restricted range of infectivity for genetically characterized stocks of the vector snail, Biomphalaria glabrata. The implication of these findings for interpretation of experimental work is clear, but one can only speculate as to whether this could result in variations in transmission that might be important epidemiologically. The phenomenon of amebocyte accumulation observed previously in certain stocks of B. glabrata have been shown to be genetically determined. They are particularly interesting becuase these accumulations of amebocytes (host-defense cells of the snail) appear to be associated with insusceptibility to infection with S. mansoni (Richards).

Filariasis: The findings from the Cook Island study in the South Pacific are all in now and in the process of final tabulation and analysis. The main clinical observations are also referred to elsewhere (LCI Report AI-00047) but included the interpretation that individuals initially exposed to filarial infection develop a good cellular immune response but become non-responsive on continued exposure. The reduced cellular responsiveness in chronic cases is specific for filarial antigens, does not include humoral antibody responsiveness, and is associated with presence of circulating microfilariae. A number of other interesting observations were made including the lack of usefulness of the filariasis skin test, results of cell-mediated immune responses before, during, and after diethylcarbamazine therapy, and eosinophil responses after therapy (Ottesen and Weller). To provide some experimental correlates to the findings in man, infections with filaria of the Brugia species were attempted and successfully established in inbred Lewis rats. Initial findings indicate that there is a sequential acquisition of cellular immune responsiveness to developing stages of the filarial parasite. In an effort to extend these observations, similar immunologic studies are being carried out simultaneously with another rodent model, the Mongolian jird (Weller, Gusmão and Ottesen).

Eosinophils in the host response to helminths: Eosinophilia has traditionally been associated with infection by helminthic parasites and LPD has been caught up, but selectively we hope, in the recent rush of immunologists

down this avenue of immune response. A technical innovation has been developed which will facilitate one part of the assay procedure for substances from lymphocytes which stimulate movement of eosinophils (ESP). This is a rapid and precise method of measuring degree of migration of cells from an agar droplet with the aid of a video-integrator. Properties of the ESP lymphokine are being investigated with the new technique (Weller and Dvorak). Joint studies are also underway with investigators from LCI on immunologic factors required to stimulate enzymes from the eosinophil in a non-cytolytic fashion (Ottesen and Metcalfe, LCI). Closely related to this work are efforts to define immunologic receptors on the eosinophil cell membrane. Preliminary evidence indicates that some cells have receptors for IgG and for complement C3b and that there may be subpopulations of eosinophils with these properties (Ottesen and Gelfand, LCI).

PATHOGENESIS AND IMMUNITY OF PROTOZOAL INFECTIONS

Toxoplasma and related coccidial organsism: The parasitologic picture and interrelationships of the expanding group of protozoa classed as coccidia continues to be murky. The latest relative that needs sorting out is the genus Sarcocystis, so various species of this parasite, must now be differentiated from many different species of Isospora, from Toxoplasma and from Hammondia. The oocyst of sporocyst, produced in the intestinal epithelium of the definitive host, may be difficult or impossible to identify specifically by usual morphological criteria. Therefore, comparative ultrastructural studies are continuing with this group. The sporozoites of each are very similar, with the exception of Toxoplasma gondii not having characteristic storage granules (Sheffield). Since cysts of one of the organisms, Sarcocystis fusiformis, are found in beef sold for human consumption, human infection via this mode of transmission is a possibility. Accordingly, serologic tests were developed which were able to detect antibody in experimentally infected cattle. The tests should be useful in seeking evidence of possible human infection and disease with the organism (Lunde). Extended observations were made on immunity in the cat at the level of the G-I tract to Toxoplasma, as reported last year. Six of the seven cats infected and treated with an anti-coccidial drug, lasalocid, over a year earlier were immune to challenge by feeding toxoplasma cysts. Immunity was judged by failure of oocyst production (Sheffield).

Trypanosomiasis: Interest in the varying susceptibility of different cells and variable infectivity of different strains of this intracellular parasite, T. cruzi, has continued at several levels. Mathematical analysis of the parasite-cell interaction with respect to penetration of cells showed differential susceptibility of vertebrate cells, as reported last year. However, susceptibility was not a stable parameter; the penetration of a host cell influenced subsequent penetrations by the parasite. The concentration of bicarbonate ion was found to influence penetration of cells by T. gondii another intracellular parasite, as well as by T. cruzi. Within a normal physiologic range there was a parabolic relationship between bicarbonate concentration on the time required for penetration (Dvorak). Differences in strains of T. cruzi that would reflect their capacity to infect and multiply in cells (virulence) has been approached by using a cell culture system to test 1) minimal numbers required to initiate infection by terminal dilution of inoculum, 2) growth curves of trypomastigotes produced by infected culture, and 3) evidence of plaques produced in infected cell sheets under agar. All

systems seem to be feasible and can be applied to trypanosomes directly from infected vector bugs (Neva). Another approach to parasite-cell interaction has been that of in vivo tissue tropism. The work referred to last year with isoenzyme elevations in the plasma of infected animals was extended to study of creatine phosphokinase and glutamyl transpeptidase as well as lactic dehydrogenases. Isoenzyme elevations indicating muscle damage were characteristic of several strains maintained in vector bugs and liver damage was seen with a laboratory passaged strain of parasite (Mercado).

Leishmaniasis: The work with the L. tropica mouse model has gone mainly in the direction of immunologic inquiry. Spread of infection to visceral organs could not be attributed to failure of at least one arm of the immune system since splenic plaque-forming cell response was actually enhanced in infected animals during the period when organisms disseminate. Only much later in the disease was the plaque-forming response depressed. Deliberate suppression of B-cell activity by an anti-B-cell serum resulted in on obvious change in the course of the infection (Weintraub and Weinbaum, LMI). Additional strains of leishmania from cases of cutaneous disease have become available which show a spectrum of biologic activity in the inbred mouse. For example, one produces virtually no lesion in the footpad and several others produce a transient lesion that regresses. The fact that these strains can be preserved in the frozen state increases their usefulness for research purposes (Neva).

Amebae and amebiasis: The role of iron in the physiology of amebae was suggested by the biochemical studies that showed presence of non-heme iron enzymes (see AI-00098-19). The amebae may have an absolute requirement for iron since presence of the metal seems essential for their growth. These findings have stimulated new attempts to develop a truly synthetic and defined medium for E. histolytica. It is tempting to speculate that the iron requirement for amebae explains their avid ingestion of red blood cells (Diamond, Harlow and Weinbach). The story of the ameba viruses becomes increasingly complicated. Three different viruses of amebae could be demonstrated in all 10 wild strains of E. histolytica tested; the icosahedral being present in all, several strains carrying two viruses, and one strain all 3 viruses. A fourth structure, resembling T-bars, is of uncertain nature. Thus far microisolated clones of amebae still contain the viruses of the parent. Although some evidence suggesting transfer of virulence by the viruses has been found this probably has resulted from selection of virulent amebae from an original population of heterogeneous virulence rather than by transfer of virulence by an integrated viral genome. For example, of four clones tested from an originally "avirulent" strain of E. histolytica, one clone was found to be virulent (Mattern of LVD, Keister, Diamond and Phillips). A new experimental animal system for testing virulence of axenically cultivated amebae has been developed. It utilizes intracerebral inoculation of neonatal animals and shows a strikingly increased sensitivity to infection. With this new animal assay for virulence perhaps it will be possible to examine the ameba-virus relationship more closely for evidence of control of virulence (Mattern of LVD, Keister and Diamond). The early events of mucosal invasion by amebae in the germ-free animal is still under study (Phillips and Takeuchi of AFIP).

VIRUS DISEASES IN THE PACIFIC AREA

Dengue: Epidemiologic studies of recent dengue outbreaks on Pacific islands have provided further evidence that severe and fatal disease can result from a first infection with a dengue virus. These observations are not in accord with the widely held belief that such disease results only from a second or third dengue infection and is the result of "sensitization" by the first such infection. The epidemiologic studies also have shown that both dengue types 1 and 2 can be propagated in nature by Aedes polynesiensis in the absence of the classical vector Aedes aegypti. In laboratory studies it was shown that Toxorhynchites amboinensis, a very large and hardy species of mosquito which does not feed on blood, was an excellent laboratory host for the detection and propagation of dengue and several other arthropod-borne viruses (Rosen and Kuberski).

Encephalomyocarditis virus: Sera from Hawaiian wild and domestic animals were examined for antibodies to EMC virus. The highest prevalence of infection was found in Rattus rattus. Neutralization tests also were performed on human sera from various regions of the world and indicated a high prevalence of EMC virus antibodies among certain Pacific island populations. Age-specific antibody rates among these groups suggest that infection is acquired in early childhood. Examination of sera from 400 juvenile diabetics and control children failed to demonstrate an association between juvenile diabetes and EMC virus infection. These studies are designed to learn more about the ecology of EMC virus and its human disease potential (Tesh, Wallace and Rosen).

Influenza virus in swine: Studies in Hawaii and on other Pacific islands are in progress to determine how swine influenza virus is maintained in nature between epidemics in swine, what factors are critical for the occurrence of an epidemic in swine, and to make observations on the interchange of influenza viruses in swine and man. Pacific islands are appropriate for such studies because of isolation and close contact between swine and humans (on some islands). Results indicate that swine influenze virus is endemic in swine in Hawaii in the absence of lungworms (in contrast to the life cycle proposed by Shope). Antibody to current human strains of influenza virus (H_3N_2) has been found in swine in a number of herds in Hawaii, but there has been no evidence that such strains have become endemic in swine (Wallace).

Influenza virus in humans: The questions of whether influenza virus is congenitally transmitted, and if so, what the consequences are to the fetus remain unanswered. To provide information on this subject, a prospective study was recently initiated during an influenza epidemic in Hawaii. In a population of women with serologic confirmation of an influenza infection isolation of virus is being attempted from serum collected during acute infection and from the placenta collected at paturition. Umbilical cord serum will be examined for specific IgM type antibody, an indication of fetal infection (Wallace and Kuberski).

Veneral Disease and Chlamydia trachomatis: Non-gonococcal urethritis (NGU) was found to be three times more common than gonorrhea in men with urethritis attending a venereal disease (VD) clinic. C. trachomatis was isolated from 42% of a series of NGU patients in this civilian VD clinic population, a finding consistent with the accumulating evidence that C. trachomatis is responsible

for a large proportion of NGU, the most common sexually transmitted disease seen in men (Kuberski).

EPIDEMIOLOGIC STUDIES ON SANDFLY FEVER VIRUS INFECTIONS

Field studies on the epidemiology of sandfly fever were carried out in Iran in an endemic region of the disease. Sixty-two isolates of Sicilian and Karimabad viruses as well as a new vesicular stomatitis virus serotype were recovered from Phlebotomus papatasi collected in the study area. Serologic studies on human inhabitants of the area indicated that many had neutralizing antibodies against these three agents. Antibodies against Sicilian and Karimabad viruses were also found in gerbil sera, suggesting that these ubiquitous rodents might serve as reservoirs or amplifying hosts of the viruses. Host identification studies on blood-engorged P. papatasi from Iran indicate that this species has a wide natural host range and is not strongly anthropophilic.

Several thousand human sera from Africa, the Mediterranean region. Central and Southeast Asia were tested for antibodies against 8 Phlebotomus fever virus serotypes known to occur in the Old World. These studies indicate that only the Naples, Sicilian and Karimabad serotypes infect significant numbers of people and are of any real public health importance (Tesh with Iranian and other collaborators).

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - July 1, 1975 through June 30, 1976

HONORS AND AWARDS

Some of the more important honors received by LPD Staff in the past year included the following:

Dr. Cheever served as a Member of the NIH Tropical Medicine and Parasitology Study Section and gave an Invited Lecture at the International Conference on Schistosomiasis in Cairo.

Dr. Diamond participated as an organizer and participant in an International Conference on Amebiasis commemorating Losch's discovery in 1875, Mexico City, October 27-29, 1975. He was also an invited participant for a seminar on viruses and plasmids in fungi and protozoa, sponsored by the American Society for Microbiologists, Atlantic City, May 4, 1976.

Dr. Dvorak was an invited speaker for an NIH Study Section sponsored Workshop on "Intracellular Parasitism".

Dr. Miller served as a Consultant to the World Health Organization and to the Agency for International Development. He served as a Member of the Ad Hoc Committee for Contract Review in Parasitic Diseases for the Walter Reed Army Institute of Research and was an invited participant to an international meeting on Vaccination Against Hemoprotozoa of Man and Amimals sponsored by the Rockefeller Foundation and an international meeting on Blood Group Antigens.

Dr. Neva was a Member of the Ad Hoc Committee for Contract Review in Parasitic Diseases for Walter Reed Army Institute of Research and was a Member of the Editorial Board of the Journal of Infectious Diseases. He was invited to participate in an international meeting in Brazil on Chagas' disease and served as Visiting Lecturer at Harvard University School of Public Health.

Dr. Richards was an invited participant for an International Symposium on "Genetical Aspects of Host-Parasite Relationships" sponsored by the British Society for Parasitology in October 1975.

Dr. Rosen received the USPHS Distinguished Service Medal; received an award for advanced research in Burma under the Fulbright-Hays Act; was appointed to the directorate of the U.S. National Program for UNESCO's Man and the Biosphere project; began his term as President of the American Society of Tropical Medicine and Hygiene; Dr. Rosen continued to serve as a member of the Ad Hoc Study Group on Viral and Rickettsial Diseases, U.S. Army Medical Research and Development Command; as a member of the Ad Hoc Advisory Group on Dengue of the South Pacific Commission; as a member of the Executive Group of the International Congress of Tropical Medicine and Malaria; as a member of the editorial board of Intervirology.

Dr. Weinbach was an invited plenary lecturer to the International Congress on Amebiasis in Mexico and an invited speaker to the Second International Symposium on the Comparative Biochemistry of Parasites in Belgium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00091-14 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Biology and Control of the Snail Intermediate Hosts of Schistosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles S. Richards	Parasitologist	LPD NIAID
Other:	Olakunle Kassim	Visiting Fellow	LPD NIAID

COOPERATING UNITS (if any)
Dr. K.J. Lie and Dr. Donald Heyneman, The George Williams Hooper Foundation, University of California. Dr. Thomas C. Cheng, Institute for Pathobiology, Lehigh University, Bethlehem, PA.

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
36/12	18/12	18/12

SUMMARY OF WORK (200 words or less - underline keywords)

Two substrains of Schistosoma mansoni have been derived by selection from our NIH St. Lucian strain, one less and one more infective than the parent strain. Preliminary tests indicate a recently isolated strain of S. mansoni of Puerto Rican origin differs genetically from our original NIH Puerto Rican strain, the new strain having a more restricted range of infectivity for genetically characterized stocks of Biomphalaria glabrata.

Crossing experiments have shown that a pathological condition in B. glabrata, with edema of the body resulting in trumpet-like flaring of the shell aperture, is inherited as a simple recessive character.

Selection and crossing have shown that amebocytic accumulations observed in certain B. glabrata stocks are genetically determined, and that there is an association between these accumulations and insusceptibility to infection with S. mansoni.

Cooperating Units Continued:

Dr. Edward H. Michelson, Department of Tropical Public Health, School of Public Health, Harvard University. Dr. Frederico S. Barbosa, Faculdade de Ciencias da Saude, Universidade de Brasilia.

Project Description:

The objective is to study molluscan and trematode genetics with particular attention to applications to control measures. To study prospective agents for biological control of intermediate hosts of the schistosomes. To study the biology of the snails with the aim of improving the efficiency of control measures. To study the contributions and complications of molluscan diseases and molluscan genetics to bio-medical research. To study the causes of tumors in mollusks. To study host-parasite interaction and molluscan cellular defence mechanisms.

Individual snails (Biomphalaria glabrata and Biomphalaria straminea) are exposed to various strains of Schistosoma mansoni, and selection and crossing experiments conducted to investigate further the genetic aspects of molluscan susceptibility to infection. Results of penetration of individual miracidia of S. mansoni in different snail types are observed and selection and crossing experiments conducted to investigate the genetic aspects of schistosome infectivity.

Previous studies involving reciprocal crosses between Puerto Rican (PR) and St. Lucian (L) S. mansoni suggested one or more of the genetic factors involved in infectivity was sex-linked. Genetic studies on infectivity in S. mansoni have resulted in selection from the parent L strain of two substrains; Lc less infective and Lt more infective than the parent strain. Reciprocal crosses between the Lc substrain and the PR strain failed to demonstrate involvement of sex-linkage.

The infectivity of a recently isolated Puerto Rican strain of S. mansoni for various genetic types of B. glabrata is being tested. Preliminary results indicate this strain has a much more restricted range of infectivity than the Puerto Rican strain which has been maintained at NIH for several years. Each strain of S. mansoni used by investigators, regardless of origin, should be considered potentially genetically different from others in infectivity, and potentially heterogenic (as demonstrated for the NIH St. Lucian strain) until proved otherwise.

S. mansoni genetic studies have involved observations of miracidial penetrations. In some of these observations, site of penetration and subsequent route of migration have been compared with location of developing primary sporocyst in each snail as observed 10 days to 2 weeks later. Most miracidia show active movement for only a few minutes after penetration. Migration appeared to be dependent on time and how compact the tissue penetrated was. Miracidia penetrating compact foot tissue tended to remain near the site of penetration while miracidia penetrating into hemolymph sinuses or vessel showed appreciable migration, some reaching and developing in the posterior kidney or even the hepatopancreas.

Establishing genetic stocks of B. glabrata, particularly different types of susceptibility to S. mansoni, provides material for bio-chemical, biological and cytogenetic intraspecific analyses. Such stocks have been provided on request to several other investigators.

Need for evaluation of the role of genetics in snail control continues. Refractory B. glabrata may prove useful in biological control of schistosomiasis by dilution or replacement of populations of susceptible snails in endemic areas. Samples of genetically refractory snails have been sent to Dr. Barbosa, University of Brasilia, to initiate collaborative laboratory and field studies along these lines. Progress in understanding causes of susceptibility or insusceptibility of B. glabrata to infection with S. mansoni accelerates the prospect of safe field testing of refractory snail stocks for control.

Establishing markers for some of the various linkage groups in B. glabrata increases our capacity to analyze invisible and complex genetic characters such as host-parasite relations. A B. glabrata with a pathological condition involving edematous swelling of the body and resultant trumpet-like flaring of the shell aperture gave rise by selfing to a clonal stock breeding true for this character. Crosses suggest it is inherited as a single recessive factor. Crosses are in progress to determine linkage characteristics.

The four clonal stocks of B. glabrata exhibiting different types of proliferative amebocytic accumulations have been subjected to an extensive combination of crosses. Results confirmed that the tendency to form the accumulations is inherited, each type being determined by a complex of genetic factors, some of which are apparently shared. Results also indicated that the association between occurrence of the accumulations and various degrees of insusceptibility to S. mansoni was not coincidental. One cross resulted in a stock of B. glabrata in which occurrence of both amebocytic accumulations and susceptibility to S. mansoni are variable. Amebocytic accumulations in this stock are observed as transitory nodules in the atrial cavity of young adult snails. The snail stock is essentially 100% susceptible to S. mansoni as juveniles. Some young adult snails remain susceptible while some are refractory. All snails exposed to S. mansoni as young adults with atrial nodules accumulations proved refractory. Most snails exposed as young adults without atrial nodules became infected. Most of the snails with atrial nodules and refractory as young adults became infected when later reexposed after the amebocytic accumulations had disappeared.

The hemopoietic organ in the anterior pericardial wall of B. glabrata was independently reported in 1975 by Lie and Heyneman of the Hooper Foundation and by LPD. This has resulted in collaborative studies on interaction between B. glabrata and S. mansoni. It has been demonstrated that when B. glabrata genetically refractory to S. mansoni are first exposed to echinostome miracidia, they lose their resistance to S. mansoni. Also collaborating in these studies, Dr. Cheng of Lehigh University is analyzing relative quantities of various enzymes in cellular and serum components of the hemolymph of B. glabrata of various genetic types, unexposed and exposed and reexposed to various combinations of echinostome and S. mansoni miracidia.

Information (including histopathology) on a variety of tumors observed in B. glabrata, many of which are genetic, should help in analysis of tumors of mollusks collected in the field. Studies on the proliferative amebocytic accumulations in B. glabrata have revealed information on hemopoiesis about which little is known, provided a possible source for molluscan tissue culture, and may help in understanding the dynamics of the early interaction between molluscan host tissues and invading miracidia of S. mansoni.

Microsporida, amebae, and bacteria cause extensive damage in some snail hosts. These and other snail diseases need to be examined for potential human infection, and prospective use as agents in biological control of snails. It is essential to be aware of the occurrence of these infections and their relations with the snails and other parasites to plan, conduct, and interpret experimental research on snails with validity.

Genetic studies on B. glabrata and B. straminea will be continued to: a) seek additional genetic markers; b) expand our knowledge of linkage groups; and c) test additional stocks of B. glabrata and strains of S. mansoni as available. Genetic studies on S. mansoni will be continued to establish different genetic infectivity strains and elucidate the genetics of infectivity.

Plans have been initiated for research studies in collaboration with Dr. Frederico Barbosa, Professor of Epidemiology at the University of Brasilia. Laboratory and field trials will be carried on in Brazil to assess the potential genetic control of transmission of S. mansoni by the use of genetic stocks of B. glabrata and B. straminea refractory to infection. The first snail shipment have been sent to Dr. Barbosa.

Studies will be continued on molluscan defence mechanisms and interaction between host snails and trematode parasites. Some of these studies are being conducted by Dr. Kassim, involving various genetic snail types and S. mansoni strains, exposures to various numbers of miracidia, reexposures at different time intervals, and exposures to more than one strain of S. mansoni. Host-parasite interactions are being studied histologically. Autoradiographic marking is being employed to differentiate between miracidia of different S. mansoni strains or sequence of penetration.

Studies on the histopathology of molluscan tumors will be continued in collaboration with Dr. Michelson.

Publications:

Richards, C.S.: Genetic studies of pathologic conditions and susceptibility to infection in Biomphalaria glabrata. Ann. N.Y. Acad. Sci. 266: 394-410, 1975.

Michelson, E.H. and Richards, C.S.: Neoplasms and tumor-like growths in the aquatic pulmonate snail Biomphalaria glabrata. Ann. N.Y. Acad. Sci. 266: 411-425, 1975.

Richards, C.S.: Dermal lesions and amebocytic accumulations in snails. Marine Fisheries Review. (in press)

Richards, C.S.: Genetics of the host-parasite relationship between Biomphalaria glabrata and Schistosoma mansoni. British Society for Parasitology Symposia. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00092-10 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The pathogenesis of schistosome infections in mammalian hosts.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Cheever, A.W. Ass't. Chief, Laboratory of Parasitic Diseases NIAID
Head, Section on Host Parasite Relations LPD NIAID

COOPERATING UNITS (if any) Southwest Foundation for Research and Education; Department of Pathology, Hospital Prof. Edgard Santos, Salvador, Brazil; Department of Pathology, Cairo University Faculty of Medicine, Cairo, Egypt; Department of Zoology, University of Georgia; Lab. Pathology, National Cancer Institute;

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-parasite relations

INSTITUTE AND LOCATION

NIAID, Bethesda

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
24/12	12/12	12/12

SUMMARY OF WORK (200 words or less - underline keywords)

Schistosome infections are studied quantitatively in man and in experimental animals. The number of adult worms present is related to passage of eggs in the excreta, accumulation of eggs in the tissues and the development of lesions in the tissues. Hepatic pathology produced by Schistosoma mansoni in Brazil and Egypt appears to be identical, both in the anatomic lesions produced and in the number of worms required to produce severe hepatic pathology. Schistosomal colonic polyposis is frequent in Egypt and extremely rare in Brazil in spite of comparable intensity of infection in these two countries. Obstructive uropathy in Egyptian patients examined at autopsy was related to the presence and intensity of S. haematobium infection; however, levels of infection were similar in cases of inconsequential and severe obstructive uropathy.

PHS-6040
(12-75)

Cooperating Units, continued:

Laboratory of Statistical and Mathematical Methodology, DCRT; Laboratory of Infectious Diseases, NIAID: Division of Georgraphic Medicine, Case Western Reserve University; Division of Clinical Oncology, University of Wisconsin Medical School.

Project Description:

The nature and pathogenesis of the lesions produced in mammalian hosts by schistosomes of medical importance are studied in man and experimental animals. Schistosome infections are quantitated by measurement of egg passage in the excreta and egg accumulation in host tissues.

Quantitative analysis of cases of schistosomiasis examined at post mortem in Egypt demonstrated the following: 1) The intensity of S. mansoni infection required to produce hepatic fibrosis and portal hypertension (Symmers' fibrosis) was the same as that in Brazil. S. haematobium infection did not produce hepatic fibrosis. In contrast to previous belief, no clinically significant liver disease other than Symmers' fibrosis was associated with infection. 2) In man, the rate of destruction of S. haematobium eggs in the tissues is much slower than that for S. mansoni. Since most pathology is related to host response to live schistosome eggs, this means that S. haematobium eggs may accumulate in the tissues in large numbers without producing significant lesions. In fact, S. haematobium eggs were as numerous as S. mansoni eggs in the livers, but were not related to Symmers' fibrosis. The same was true for the lung, where S. haematobium eggs were present in much higher numbers than S. mansoni, but pathologic changes were primarily related to S. mansoni eggs. 3) Pyelonephritis secondary to schistosomal obstructive uropathy was related entirely to bladder outlet obstruction and distortion of the bladder trigone with disruption of the ureterovesical junction. In the absence of these lesions, marked hydroureter did not predispose to pyelonephritis. 4) The presence of obstructive uropathy was related to the presence and intensity of S. haematobium infection; however, cases with mild and severe obstructive uropathy had similar levels of infection. Obstructive uropathy was usually caused by primary schistosomiasis of the ureter. It would appear that the specific location of lesions is more important than the overall intensity of infection in the pathogenesis of obstructive uropathy. 5) Schistosomal colonic polyposis is one of the severe manifestations of S. mansoni infection in Egypt, but is rarely seen in the western hemisphere. Our data confirmed the relation of colonic polyposis to intensity of S. mansoni infection but also showed that the intensity of infection in Brazilian cases was as high as that in Egyptian cases, and the distribution of eggs (e.g. between colon and liver) was also similar. 6) Egg excretion in the urine and feces roughly paralleled the number of worm pairs present for both S. haematobium and S. mansoni, supporting the general validity of quantitative examination of excreta in living patients. No diminution of worm fecundity was seen with increasing age. In animals demonstrating immunity to schistosomes, diminished worm fecundity is one of the most sensitive indicators of immunity.

Baboons chronically infected with S. mansoni showed partial immunity to reinfection, diminished worm fecundity and migration of worms from colonic to small intestinal venules. Pathologic changes did not resemble chronic lesions in man. The model appears to be useful for investigation of immunobiologic aspects of schistosomiasis, which resemble those in man.

Capuchin monkeys reinfected with S. haematobium also demonstrate partial immunity to reinfection. Many of the reinfecting worms appear to be killed as adults, which is at variance with current dogma concerning concommitant immunity in schistosome infection.

Study of the pathology produced by temperature sensitive mutants of Streptococcus pneumoniae are in progress with Drs. Helms and Grizzard of LID, NIAID.

During the next year we will analyze in detail the pulmonary lesions in Egyptian patients and their relation to cor pulmonale. Preliminary examination suggests that chronic bronchitis is by far the most significant pulmonary disease, and that this is not related to schistosome infection. A hepatic lesion believed to be the precursor of Symmers' fibrosis of the liver has been identified, and the morphology of this lesion needs to be examined in detail. Bladder cancers in monkeys with S. haematobium infection will continue to be followed. Many monkeys have now been followed for over 3 years, and none has been shown to be invasive by the usual criteria. Penetration of the bladder basement membrane by tumor has been seen ultrastructurally.

Understanding of the lesions cause by schistosomiasis in man, the associated morbidity and their relation to intensity of infection are basic to planning control of the disease and to treatment of patients. If light infections are unimportant, then these should not be treated (all available drugs are toxic) and control measures aimed at reducing infection intensity should be vigorously supported.

Publications:

Cheever, A.W., Young, S.W., and Shehata, A.: Calcification of Schistosoma haematobium eggs: relation of radiologically demonstrable calcification to eggs in tissues and passage of eggs in urine. Trans. Roy. Soc. Trop. Med. Hyg. 69: 410-414, 1975.

Mahmoud, A.A.F., Cheever, A.W., and Warren, K.S.: Streptozotocin-induced diabetes mellitus and the host-parasite relation in murine schistosomiasis mansoni. J. Inf. Dis. 131: 634-642, 1975.

Sadun, E.H., Reid, W.A., Cheever, A.W., Duvall, R.H., Swan, K.G., Kent, K.M., Bruce, J.I., and von Lichtenberg, F.: Effects of portacaval shunting on Schistosoma japonicum infection in chimpanzees: dissociation of pipestem fibrosis and glomerulopathy. Am. J. Trop. Med. Hyg. 24: 619-631, 1975.

Damian, R.T., Greene, N.D., Meyer, K.F., Cheever, A.W., Hubbard, W.J., Hawes, M.E., and Clark, J.D.: Schistosoma mansoni in baboons, III. The course and characteristics of infection with additional observations on immunity. Am. J. Trop. Med. Hyg. 25: 299-306, 1976.

Cheever, A.W., Kuntz, R.E., Bryan, G.T., Brown, R.R., and Moore, J.A.: Carcinoma of the urinary bladder in association with Schistosomiasis haematobia. Am. J. Path. (in press)

Sadigursky, M., Kamel, I.A., Elwi, A.M., Danner, R., Cheever, A.W., and Andrade, A.Z.A.: Absence of schistosomal glomerulopathy in Schistosoma haematobium infection in man. Trans. Roy. Soc. Trop. Med. Hyg. (in press)

Cheever, A.W., Elwi, A.M., Kamel, I.A., Mosimann, J.E. and Danner, R.: Intensity of infection as related to pathological lesions in bilharzial patients. Proc. Internat. Conf. Schistosomiasis. (in press).

Cheever, A.W., Kuntz, R.E., Moore, J.A. and Huang, T.C.: Proliferative epithelial lesions of the urinary bladder in cynomolgus monkeys (Macaca fasicularis) infected with Schistosoma intercalatum. Cancer Research (in press).

McCully, R.M., Barron, C.N., and Cheever, A.W.: Schistosomiasis (Bilharziasis). in Connor, D. and Binford, C. (eds): Atlas of Pathology of Tropical Diseases. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00093-02 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of the Immunologic Response to Helminth Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eric A. Ottesen	Senior Investigator	LPD NIAID
Other:	Peter F. Weller	Research Associate	LPD NIAID
	Renato Gusmao	Visiting Fellow	LPD NIAID
	Allen W. Cheever	Assistant Chief	LPD NIAID
	Franklin A. Neva	Chief	LPD NIAID
	Robert W. Gwadz	Research Entomologist	LPD NIAID
	Dean D. Metcalfe	Clinical Associate	LCI NIAID
	Jeffrey A. Gelfand	Clinical Associate	LCI NIAID

COOPERATING UNITS (if any) Duke University Medical Center (Dr. B. Amos, Dr. F. Ward). San Juan Laboratory, Center For Disease Control (Dr. R. Hiatt); University of Puerto Rico (Dr. Z.R. Sotomayor); Laboratory of Clinical Investigation, NIAID, Division of Computer Research and Technology, NIH.

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
52/12	33/12	19/12

SUMMARY OF WORK (200 words or less - underline keywords)

The major goals of this project are to characterize the host's immune response to helminth infections and to relate these findings to the pathogenesis of clinical disease.

Field studies of bancroftian filariasis suggest that initial exposure to these parasites elicits vigorous cellular immune responses but with continued reexposure, infected individuals cannot manifest significant cellular reactivity to filaria products. This defect in responsiveness is filaria-specific and limited to cellular immunity.

Similar findings come from studies of human schistosomiasis mansoni. Patients evaluated within three months of initial infection demonstrated marked cellular reactivity to schistosome antigens, whereas lymphocytes from those with chronic infection were unable to respond to schistosome products.

Studies of the cellular responses to helminth infections by lymphocytes and by eosinophil leukocytes as well as the mechanisms underlying the diminished reactivity to parasite antigens found in schistosomiasis and filariasis will continue both in experimental animals and in man.

Project Description:

The character and evolution of the immune response in man and experimental animals is studied during and following infection with parasitic helminths.

Human filarial infection. In a field study of bancroftian filariasis (Project #J0233-I-74) the major difference found between the immune responses of infected and non-infected individuals was the presence of strong cellular immunity to filarial antigens (measured by lymphocyte transformation) in the exposed, non-infected population but its almost complete absence among infected individuals. This defect in immune responsiveness was specific for filarial antigens and limited to cellular immunity, as other aspects of the host response (eg. cellular reactivity to non-filarial antigens, skin-test findings, immunoglobulin levels, specific antibody titers, complement levels) were not remarkably different between the two groups. (Ottesen & Weller).

Filarial infection in rodents. Attempts to establish laboratory infections with filaria of the Brugia species in inbred Lewis rats have been successful. Initial findings suggest that there is a sequential acquisition of cellular immune responsiveness to developing stages of the filarial parasites. Corresponding antibody responses are being evaluated. In an effort to extend these observations, similar immunologic studies are being carried out simultaneously with another rodent model, the mongolian jird with Brugia pahangi filariasis. (Weller, Gusmao, Ottesen).

Human schistosomiasis. Patients with early (10-12 weeks), recent (9-12 mos) and chronic (many years) schistosomiasis mansoni have been evaluated jointly at the NIH and the University of Puerto Rico. In the earliest phases of the disease studied all patients demonstrated marked cellular immune reactivity (lymphocyte transformation) to schistosomal antigens prepared from all three major developmental stages seen by man - cercaria, adult worm, and egg. Chronically infected individuals manifested no such cellular responsiveness to schistosomal antigens despite their normal responsiveness to other environmental antigens. Correlations of these immunologic parameters with the clinical aspects of disease (eg. symptoms, pathology, and intensity of infection) is in progress. (Ottesen, Cheever, Neva).

Eosinophils in the host response to helminths. A rapid, effective video-integrator technique for the assay of the lymphocyte product 'eosinophil stimulation promoter' (ESP) has been developed (Weller, Dvorak) and employed with techniques of column chromatography, enzyme digestion, metabolic inhibition, etc. to determine the biophysical and biochemical characteristics of the human lymphokine ESP. In supernatants of antigen-stimulated leucocyte cultures, eosinophil stimulating activity was found in three discrete molecular fractions-one in the region of 30,000 - 60,000 dalton molecular weight, one in the 3,500 range, and another in the < 1,000 region. The nature and specificity of these active fractions is still under investigation. (Weller). Other studies on the eosinophil include two joint projects with investigators from the Laboratory of Clinical Investigation, NIAID. In the presence of schistosome antigen and specific antibody (with or without complement) human eosinophils from patients with schistosomiasis are stimulated to release their enzyme stores

(specifically, aryl sulfatase) in a non-cytolytic fashion. (Metcalfe, Ottesen). To determine the mechanisms through which this and other eosinophil responses take place, it is necessary to understand the nature of the eosinophil cell membrane and the types of receptors it bears. Initial studies of patients with parasitic helminth infections have demonstrated the presence of receptors for 7S immunoglobulin and the complement C3b on between 25% and 50% of these cells. The nature and meaning of these subpopulations of eosinophils is currently under study. (Ottesen, Gelfand).

Publications:

Ottesen, E.A., Smith, T.K., Kirkpatrick, C.H.: Immune Response to Trichinella spiralis in the Rat. I. Development of Cellular and Humoral Responses During Chronic Infection. Int. Arch. Allergy 49: 396-410, 1975.

Ottesen, E.A.: Eosinophilia and the Lung; in Kirkpatrick, C.H., and Reynolds, H.Y. (eds): Immunologic and Infectious Reactions in the Lung. M. Dekker, Inc., New York, 1976, pp. 289-332.

Levin, D.M., Ottesen, E.A., Reynolds, H.Y., Kirkpatrick, C.H.: Cellular Immunity in Peyer's Patches of Rats Infected with Trichinella spiralis. Infection and Immunity 13: 27-30, 1976.

Kirkpatrick, C.H., Ottesen, E.A., Smith, T.K., Wells, S.A., and Burdick, J.F.: Reconstitution of Defective Cellular Transfer Factor. Clin. Exp. Immunol. 23: 414-428, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ZI 00094-17 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Entamoeba histolytica and Other Parasitic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. S. Diamond	Head, Parasite Growth and Differentiation Section	LPD NIAID
Other:	D. B. Keister	Biologist	LPD NIAID
	B. P. Phillips	Research Biologist	LPD NIAID
	D. Harlow	Senior Staff Fellow	LPD NIAID
	E. C. Weinbach	Head, Physiology and Biochemistry Section	LPD NIAID
	C. F. T. Mattern	Medical Officer	LVD NIAID

COOPERATING UNITS (if any)

Laboratory of Viral Diseases, NIAID

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasite Growth and Differentiation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
60/12	30/12	30/12

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year research efforts under this project were concentrated on Entamoeba histolytica in three study areas: 1) nutrition of the amebae in axenic culture, 2) mechanisms involved in production of amebal lesions in experimental animals and 3) the endogenous viruses of this parasite, their further characterization, and the role they may play in influencing amebal virulence.

These studies will be continued next year. Additionally, special attention will be given to development of a medium for isolation of Giardia lamblia from human feces.

Project Description:

During the past year all research efforts were concentrated on Entamoeba histolytica in 3 study areas: 1) nutrition of the amebae in axenic culture, 2) mechanisms involved in production of amebal lesions in experimental animals, and 3) the endogenous viruses of this parasite, their further characterization, and the role they may play in influencing amebal virulence.

Problems associated with the manufacture of uniform batches of Panmede, an ox-liver digest and a principal ingredient of TP-S-1 the standard medium for axenic cultivation of E. histolytica led to search for an ingredient(s) to replace this product and eventually to develop a defined medium. In the course of these investigations a requirement for iron was demonstrated which could be met equally through addition of ferritin or ferric ammonium citrate and to a lesser extent by freeze-lysed rabbit blood to the medium. Ferritin very likely is one of the principal forms of iron available in Panmede. This finding is not unexpected in view of the proven need for iron in a wide variety of organisms and our own collaborative studies with E. C. Weinbach, H. Kon and C. E. Claggett (see ZO1 AI 00098-20 LPD) which demonstrated the presence of iron-sulfur proteins in E. histolytica and their participation in electron transport reactions. Additionally, analysis of the amebae by energy dispersive X-ray spectrometry (Diamond, Harlow and C. Phillips of the Finnigan Corp. California) showed that the amebae concentrate iron in quantity. This great demand for iron may help to explain why amebal trophozoites in man and axenic culture avidly ingest erythrocytes.

The demonstration by several investigators that iron is involved in the pathogenesis of microbial diseases and the results of our own studies on the role of iron in the nutrition and metabolism of E. histolytica led us to examine the possible role of this element in amebic infections. Using young adult golden hamsters in an animal model system, wherein axenically cultivated E. histolytica are introduced directly into hepatic tissue via laparotomy, we showed that the intraperitoneal administration of ferric ammonium citrate in divided doses (24 hours prior to, coincident with amebal inoculation, and 24 hours later) resulted in a significant enhancement of the severity of the hepatic lesions, at least twice that of the untreated controls. This study, preliminary in nature, will be followed by others to determine such optima as iron dosage and time of iron administration relative to inoculation of the amebae, the influence of iron on amebal strains exhibiting varying degrees of virulence and the mechanisms through which iron acts in the production of lesions. (Diamond, Phillips, Keister and Harlow).

The animal model systems presently employed for the study of experimental amebiasis are far from ideal. The majority of their shortcomings stem from the use of animals of weanling age or older and the frequent need of having to place these animals on special diets in order to insure production of lesions with some degree of regularity. Studies first initiated by C. F. T. Mattern have led to the development of two new animal models which overcome many of the deficiencies of the earlier ones. Both employ neonatal animals and axenically cultivated amebae.

In one system the amebae are inoculated intracerebrally into day-old mice. Amebic invasion of the brain tissue with round cell infiltration and multiple abscess formation rapidly ensues, with death occurring in 3 to 20 or more days depending on the number of amebae introduced and the virulence of the strain. As few as 20 amebae from a virulent strain produce abscesses. Readily observable abnormal neurological signs in the mice parallel progressing brain damage. Three or 6 week-old mice are increasingly resistant to infection. Of special interest, these older mice inoculated with virulent amebal strains or newborn mice inoculated with strains of low virulence often develop hydrocephalus 2 to 5 weeks after inoculation. The pathology of this disease is under study.

In the other animal model, newborn hamsters which are highly susceptible to amebae introduced intrahepatically are utilized. Twenty amebae from a virulent strain (HM-1) can produce hepatic abscesses within 10 days, and with increasing doses the percentage of animals becoming infected rises until an incidence of 100% in attained with 2,000 amebae. In contrast, to achieve a similar incidence using young adult hamsters the standard animal model for hepatic amebiasis would require an inoculum of 10 million amebae. Incidentally, studies show 7 day-old hamsters exhibit significant resistance to infection.

A special advantage of this system is that the fine skin texture of the neonatal hamster permits ready visualization of the liver so that inoculation of the amebae can be made directly through the body wall obviating the laporotomy which must be employed when using older animals. Furthermore, hepatic lesions developing on the anterior surface of the liver can be seen through the skin as early as 4 days after inoculation. The lesions produced in the neonatal hamster are of the classical necrotic type found in adult hamsters and contain numerous motile amebae (Mattern, Keister and Diamond). (see also Z01 AI 00015-09 LVD).

Preliminary data obtained first through use of the adult hamster and later the neonatal hamster suggests a relationship between the endogenous viruses of E. histolytica and virulence of the organism. Strain HK-9 amebae are practically avirulent in the hamster liver, at least 15 million being required to produce a minimal sized lesion in the adult and 20,000 in the neonate. However, cultures grown from survivors after infection with the viruses of strain HM-1 amebae (high virulence potential) manifested in both adults and neonates a virulence potential several fold that of the parental HK-9 in that they required significantly fewer organisms to produce lesions. Lesions were larger and the incidence of infection greater. Furthermore, HK-9 survivor cultures resulting after infection with the virus of strain HB-301 (low virulence potential) when introduced into neonates exhibited a virulence distinctly greater than either the HB-301 donor or parental HK-9. Expecially significant was the finding that both types of survivor cultures were resistant to superinfection with the donor viruses indicating they were carrying these viruses.

At present two possible explanations can be offered for the observed results. The heterologous virus infection may be selecting for virulent amebae in a heterogenous population, or lysogeny exists and the integrated viral genome

alters the amebal virulence. Available data from studies of HK-9 clones favors a selection process. Of 4 clones tested in neonatal hamsters, 2 were avirulent, one slightly virulent and one about as virulent as the wild survivor culture. Future studies will investigate a number of clones representing additional strains and a variety of survivor cultures in an attempt to establish the basis for viral alteration of virulence. (Mattern, Keister, Diamond and Phillips).

The beaded entity first reported three years ago in E. histolytica has been studied in sufficient detail to permit it being considered a virus. It can be passaged continuously in susceptible amebal hosts causing lysis and passes through 0.2 nm filters. It develops intranuclearly and time studies indicate that it replicates within 12 hours after infecting the host. The virus assembles as large ordered arrays of parallel packed beaded rods (300 nm in length). Isolated particles observed by negative staining are usually 300 nm long and consist of 14 beads. Particles 600 nm long consisting of 28 beads (apparently dimers) are also seen. To-date the virus has been isolated from 4 to 10 amebal strains. (Mattern, Diamond and Keister).

Electron dense bars frecuently arranged in the form of rosettes have been described in EM studies of several species of Entamoeba. We have observed these bars undergo morphological differentiation in axenically cultivated E. histolytica which have degenerated either spontaneously or after infection with heterologous amebal virus. The bars develop a flattened circular plate perpendicular to the bar's axis. These differentiated bars have a unique appearance resembling bolts and have been named T-bars. Masses of T-bars have been found in phagocytic vacuoles of healthy amebae suggesting fresh ingestion. In other cases single T-bars have been found in small vacuoles attached to the membrane by the differentiated end and appear to be extruding their internal contents. The bars bear a slight resemblance to bacteriophage. (Mattern, Diamond, Daniels, and Keister).

Determination of the amebal host range of the endogenous viruses of E. histolytica has been completed for 10 wild strains and clones of three of these strains. The significant findings are as follows: All strains carried one or more viruses to which they themselves were resistant and which, with one exception, could infect two or more recipients. An icosahedral virus was present in all strains, 2 strains additionally harbored filamentous virus, and 4 strains beaded virus. One carried all three. Regardless of donor source the viruses within a group were morphologically indistinguishable. However, host range indicated the existence of more than one virus within each group. The viruses varied widely with respect to host range. A sensitive strain did not necessarily reproduce the total viral complement of a donor with multiple agents. Limited studies with the clones indicated that the clones carried the viral complement of the parent and that the host range of the viruses was similar to those of the parents. (Diamond, Mattern, Keister and Daniels).

Publications:

Takeuchi, T., Weinbach, E.C. and Diamond, L.S.: Pyruvate oxidase (CoA acetylating) in Entamoeba histolytica. Biochem. Biophys. Res. Commun. 65: 591-596, 1975.

Weinbach, E.C., Diamond, L.S., Claggett, C.E. and Kon, H.: Iron-sulfur proteins of Entamoeba histolytica. J. Parasitol. 62: 126-128, 1976.

Harlow, D.R., Weinbach, E.C. and Diamond, L.S.: Nicotinamide nucleotide transhydrogenase in Entamoeba histolytica, a protozoan lacking mitochondria. Comp. Biochem. Physiol. 53B: 141-144, 1976.

Diamond, L.S., and Mattern, C.F.T.: Protozoal viruses. In Advances in Virus Research. 20: 87-112. Academic Press, Inc., New York, 1976.

Diamond, L.S., Mattern, C.F.T., Bartgis, I.L., Daniel, W.A., and Keister, D.B.: Viruses of Entamoeba histolytica VI. A study of host range. Proc. Internat'l Conference on Amebiasis, Mexico City, Oct. 1975. Eds. Sepulveda, B. and Diamond, L.S. (in press).

Mattern, C.F.T., Diamond, L.S., Daniel, W.A., and Keister, D.B.: Unusual variation of "normal" cytoplasmic bars of Entamoeba. Proc. Internat'l Conference on Amebiasis, Mexico City, Oct. 1975. Eds. Sepulveda, B. and Diamond, L.S. (in press).

Weinbach, E.C., Harlow, D.R., Takeuchi, T., Diamond, L.S., Claggett, C.E. and Kon, H.: Aerobic metabolism of Entamoeba histolytica: Facts and Fallacies. Proc. Internat'l Conference on Amebiasis, Mexico City, Oct. 1975. Eds. Sepulveda, B. and Diamond, L.S. (in press).

Weinbach, E.C., Harlow, D.R., Claggett, C.E. and Diamond, L.S.: Entamoeba histolytica: Diaphorase activities. Exp. Parasitol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00096-02 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pathobiologic Investigation of Parasitic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	*Bruce P. Phillips	Research Biologist	LPD NIAID
Other:	**Louis S. Diamond	Research Zoologist	LPD NIAID
	*Akio Takeuchi	Chief, Lab. Exp. Path.	WRAIR-WRAMC
	*Helen Jervis	Research Physiologist	WRAIR-WRAMC

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasitic Growth and Differentiation

INSTITUTE AND LOCATION

NIAID, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13/12	12/12	1/12

SUMMARY OF WORK (200 words or less - underline keywords)

*It is the purpose of this project to study the pathogenesis of intestinal amebiasis, sequentially, at the ultrastructural level, to ascertain the mechanism of epithelial penetration by Entamoeba histolytica and to visualize and and describe the cellular interactions and effects of ameba on organelles of all intestinal tissue cells and wandering inflammatory cellular elements, from the time ameba become established in the gut lumen through the process of ulcer formation.

**It is the purpose of this sub-project to compare the virulence of various strains of Entamoeba histolytica by intrahepatic inoculation of golden hamsters, to make histopathological evaluations and comparisons on the effects of such inoculations and to determine the relevance of certain Endogeous viruses to the pathologic processes and virulence of particular strains of ameba.

Project Description:

*OBJECTIVES

To provide documented information on the mechanisms of pathogenesis in intestinal amebiasis including the mechanisms of epithelial penetration by ameba, the effect of amebae on all cellular elements and their organelles, the role of cellular interactions in the pathogenesis process, the time of appearance and the specific nature of the inflammatory process and the sequence and severity of vascular changes occurring in the disease.

PROCEDURES

Germfree guinea pigs are inoculated intracecally with E. histolytica and the enteric flora from patients with acute amebic colitis. Examination of grossly normal tissues by electron microscopy, in animals sacrificed at post-inoculation intervals of a few hours to 14 days, reveals the sequence of events attending amebic invasion of intestinal tissues and providing information relative to the mechanisms of pathogenesis.

OBSERVATIONS

The present studies indicate that the pathogenesis of acute amebic enteritis involves a very complex yet orderly and consistent series of physiological events that begin with amebae confined to the intestinal lumen. The earliest evidence of apparent toxic activity by ameba is a change in the microvilli of epithelial cells in close proximity to lumen amebae. This is followed closely by changes in the intracellular components of these epithelial elements i.e. mitochondria become swollen, the cristae deranged and endoplasmic reticulum becomes dilated. Epithelial cells, after having, undergone complete disappearance of microvilli, often project their smooth plasma membrane into the lumen contacting amebae. Such cells become detached from their basement membranes and lateral junctions and are extruded into the lumen thus causing gaps or discontinuity of epithelium through which amebae move by their pseudopodia into the mucosa and progress into the sub-epithelial tissue of the lamina propria.

Polymorphonulcear lukocytes (PMN) begin emigrating from the capillary lumen and appear in the lamina propria as soon as ameba invade epithelial tissue. By the time amebae reach the lamina propria, PMN, in large numbers, are in evidence in areas of invading amebae. These PMN begin to show a variety of degenerative changes including depletion of glycogen particles, condensation of cytoplasm, and disruption of their cell membranes with release of cytoplasmic contents into the environ.

When amebae appear in the lamina propria, the lumina of capillaries and venules have become dilated and filled with RBC, PMN, and aggregations of platelets. These vasculatures also begin to show various degrees of endothelial changes such as swelling and loss of cytoplasmic density, and dilation

and swelling of endoplasmic reticulum and mitochondria. Such thrombotic processes occur at cell junctions and at the fenestrae. In some vascular tissues, endothelial cells become separated from their basal lamina and some cells develop multiple vesicles of uniform size which appear to be derived from the cell membrane. These are but a few of the cellular interactions that have been documented. Some have been reported by us and others will be reported in future publications along with details of developing changes in intestinal enzymes as a result of mucosal invasion by amebae.

SIGNIFICANCE

After a century of considerable international speculation and hypothesizing on the mechanisms operative in the pathogenesis of amebic enteritis, these studies document, at the ultrastructural level, the complex nature of the cellular interactions attending mocosal invasion by ameba, -cellular interactions that facilitate the invasive process. Previously, data within this area was limited to either light microscopic observations or EM observations of advanced stages of the disease that tended to by-pass the early cellular interactions which we believe provide the key to pathogenesis of amebic enteritis.

FUTURE

We project that by the end of the current calendar year or a little later, we will have observed and described the whole spectrum of pathogenesis of intestinal amebiasis, ultrastructurally and sequentially, including cellular interactions, from the time of initial lumen infection by ameba through ulcer formation. At that time the principle investigator will be leaving NIAID and the project will be terminated.

**Pertinent information relative to the collaborative work on amebiasis in hamsters is presented elsewhere by the senior investigator on that project, Dr. Diamond.

PUBLICATIONS:

Phillips, B.P., and Zierdt, C.H.: Blastocystis hominis: Studies on its pathogenicity in human patients and in gnotobiotes. Exp. Parasitol. (In press).

Diamond, L.S., Phillips, B.P., and Bartigis, I.L.: The clawed jird (Meriones ugiculata) as an experimental host for the study of hepic amebiasis. Arch. de Investigacion Medica 5: (2) 465-470, 1974.

Takeuchi, A., and Phillips, B.P.: Electron microscope studies of experimental Entamoeba histolytica infection in the guinea pig. II. Early cellular and vascular changes accompanying invasion of the lamina propria. Virchows Arch B. Cell. Path. 20: 1-13, 1976.

Takeuchi, A., and Phillips, B.P.: Interactions of Entamoeba histolytica with host cells in the gut muchosa. Proc. Int. Symp. Amebiasis, Mexico City, Mexico. 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00097-18 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pathological Physiology and Histochemistry of Parasitic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Teresa I. Mercado Research Physiologist LPD NIAID

COOPERATING UNITS (if any)

Comparative Pathology Section, Division of Research Services (Dr. Anton M. Allen)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
26/12	12/12	14/12

SUMMARY OF WORK (200 words or less - underline keywords)

Knowledge of the pathological physiology of parasitic diseases and of differences between parasitic strains is advanced by studying the susceptibility of host tissues to parasitic invasion and assessing the alteration of various physiological and histochemical parameters. On the basis of significant increases in total plasma creatine phosphokinase (CPK), Y-glutamyl transpeptidase, and CPK isoenzymes, the present findings clearly demonstrate marked muscle damage in infections with the House 510 and House 11 strains of Trypanosoma cruzi and significant deterioration of liver in infections with the Tulahuen strain. The occurrence of at least one additional isoenzyme, more cathodic than LDH-5, identified as LDH-X, was associated with testicular and epididymal tissues. Corresponding histopathological changes indicating injury in these organs were observed. These findings indicate the usefulness of isoenzyme studies in assessing tissue injury in parasitic infections and in the differentiation of parasitic strains e.g., myotropic vs reticulotropic strains of T. cruzi.

PHS-6040
(12-75)

Project Description:

Tissues from mice infected with Trypanosoma cruzi strains of different origins were processed histologically to assess parasitic invasion and pathologic changes. The extent of physiologic injury was determined by assaying plasma and tissue isoenzymes employing biochemical and histochemical techniques, disc gel electrophoresis, planimetry, and densitometry. T. cruzi trypomastigotes were isolated from the blood of infected mice by ion exchange chromatography. Quantitative assays of total plasma creatine phosphokinase (CPK) an enzyme occurring predominantly in heart, skeletal muscle, and brain, disclosed increases of 79 and 66 times the normal level in mice acutely infected (10-14 days) with the House 510 (Costa Rica) and House 11 (Nicaragua) strains. Correspondingly, wide, intensely stained CPK-MM (skeletal muscle), CPK-MB (heart), and CPK-BB (brain) profiles of isoenzymes derived from plasma were observed. Mice infected with the Tulahuen strain (acute, 6-day infections) did not exhibit marked deviations from the normal pattern, but the intensity of some bands was increased; e.g., CPK-MM, which was rarely seen in uninfected plasma. Similarly, total plasma CPK levels, 4 times the normal level, were not as highly elevated. By contrast, total plasma levels of Y-glutamyl transpeptidase, a liver-specific enzyme, were higher in the Tulahuen infections and no deviations from the normal level were exhibited either in the House 510 or House 11 infections. Further studies with these strains of lactate dehydrogenase isoenzymes (LDH) revealed at least one additional isoenzyme, cathodic to LDH-5, which originates in testicular and epididymal tissues. This enzyme, identified by many workers as LDH-X, is distinct from the other LDH isoenzymes and appears to be specific for spermatozoa and spermatogenic cells. Assays employing (dl) alpha-hydroxyvaleric acid, a more specific substrate than (dl) lactic acid, disclosed its presence only in infected plasma. Corresponding histopathological changes involving disorganization and narrowing of the testicular cellular layers and some necrosis were observed. As was observed with the House 510 strain, trypomastigotes of the Tulahuen and House 11 strains, isolated from infected blood, exhibited one LDH isoenzyme which migrated near LDH-5. These findings indicate the usefulness of isoenzyme studies in assessing tissue damage during parasitic infections, and based on the detectable increases of particular isoenzymes, aid in the differentiation among strains e.g., myotropic (House 510 and House 11) vs reticulotropic (Tulahuen) strains.

Basic cellular studies provide a means for understanding the physiological mechanisms involved in the response of the host to parasitic infections and may lead to the identification of specific parasite substances involved in pathogenesis. Histochemical studies aid in localizing the altered sites within tissues. Physiological studies quantitate the alteration of activity observed histochemically and aid in defining further the physiological mechanisms that may be damaged. Basic studies on strain differentiation aid in taxonomic classification and in assessing the pathogenicity of a parasitic infection.

Studies on creatine phosphokinase and LDH-X have been completed and the manuscripts are being prepared for publication. Isoenzyme studies with malate dehydrogenase and isolated T. cruzi trypomastigotes will be continued.

Publications:

Mercado, T.I.: Trypanosoma cruzi: Lactate dehydrogenase isoenzymes and infections in mice. Exper. Parasitol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00098-20 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical mechanisms of energy metabolism in mammalian and parasitic organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	E. C. Weinbach	Head, Section on Physiology and Biochemistry	LPD NIAID
Other:	L. S. Diamond	Research Zoologist	LPD NIAID
	D. R. Harlow	Senior Staff Fellow	LPD NIAID
	T. Takeuchi	Visiting Fellow	LPD NIAID

COOPERATING UNITS (if any)

Laboratory of Chemical Physics, NIAMDD (Hideo Kon)
Laboratory of Streptoccal Diseases, NIAID (Theodore Theodore)
Metabolism Branch, NCI (Donald Tschudy)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
32/12	18/12	14/12

SUMMARY OF WORK (200 words or less - underline keywords)

As part of a broad program to study aerobic energy metabolism in parasites and mammals, current interest is focused on the role of iron-sulfur proteins in the respiratory chain of Entamoeba histolytica. Chemical analyses of a partially purified amebal diaphorase (which catalyzes the oxidation of NAD(P)H with molecular oxygen as final electron acceptor) disclosed the presence of non-heme iron and acid-labile sulfide in a molar ratio of 1:1. When examined by low-temperature electron parametic resonance (EPR) spectroscopy, the diaphorase exhibited an unusually symmetrical, narrow signal at g = 2.036. The EPR spectra of diaphorase preparations isolated from amebae grown with ^{57}Fe showed a broadened signal, and analyses by mass spectroscopy confirmed that isotopic substitution had occurred, thus providing unequivocal evidence that the EPR signal arises from an iron-sulfur center in the amebal diaphorase. Other studies demonstrated that non-heme iron also functions in the bacterial respiratory chain. Mammalian studies included a collaborative investigation of the interrelationship of heme synthesis and mitochondrial bioenergetics. Future work will attempt to further purify, characterize and elucidate the role of iron-sulfur proteins in electron and energy transfer reactions.

Project Description:

The objective of this project is to conduct fundamental studies on the mechanism of aerobic energy metabolism in mammalian and parasitic organisms. Currently, these studies center primarily on mechanisms of terminal electron transfer reactions and oxidative phosphorylation.

Tissues and cells are disrupted mechanically and subcellular fractions are isolated by differential centrifugation. Cellular and subcellular constituents, enzymatic activities and metabolic pathways are determined, characterized and elucidated by enzymatic, chemical, radiochemical and physical methods. Oxygen consumption is determined polarographically with the Clark oxygen electrode.

Parasite studies: Continued investigation of the respiratory chain in Entamoeba histolytica, strain HK-9, cultivated axenically in Diamond's liquid medium, was focused principally on the iron-sulfur electron transfer proteins. Considerable effort was devoted to the partial purification of the amebal diaphorase, which catalyzes oxidation of NAD(P)H with oxygen as the terminal electron acceptor. An 18-fold increase in specific activity was obtained by acetone and ammonium sulfate precipitations, column chromatography and thermal coagulation of inert proteins. Chemical analyses of the purified diaphorase disclosed the presence of non-heme iron and acid-labile sulfide in a molar ratio of 1:1, characteristic of all iron-sulfur proteins studied thus far. The diaphorase when examined by low-temperature electron paramagnetic resonance (EPR) spectroscopy in collaboration with Dr. Kon, NIAMDD, exhibited an unusually symmetrical, narrow (peak-to-peak width of 25 gauss) signal at $g = 2.036$. The signal was incrementally intensified upon titration with NADPH, and abolished after acidification, which released H_2S. EPR spectra of the purified diaphorase prepared from amebae grown in the presence of $^{57}FeCl_3$ showed a broadened signal at $g = 2.036$ (done in collaboration with Dr. Harlow). Analyses of the preparation by mass spectroscopy disclosed that 30% isotopic substitution had occurred, thus providing unequivocal evidence that the EPR signal arises from an iron-sulfur center in the diaphorase protein.

In collaboration with Dr. Takeuchi, studies were continued on the newly discovered enzyme, pyruvate oxidase (Co A acetylating) reported last year, and now officially recognized by the International Union of Biochemistry. The oxidase was purified by ammonium sulfate precipitation, hydroxyapatite and aminoethyl cellulose chromatography. Pyruvate oxidase contains non-heme iron and is probably iron-sulfur protein. NADPH-diaphorase was found to be closely associated with pyruvate oxidase; every fraction that contained the oxidase also contained the diaphorase. Further characterization of the diaphorase indicates the presence of two distinct non-heme iron centers; one gives the characteristic EPR signal, the other does not. Both the oxidase and the diaphorase exhibit complex patterns of multiple forms. At least eight different forms of pyruvate oxidase and NADPH-diaphorase were identified by their elution profiles from column chromatography.

Flavoproteins, identified by fluorometry and difference spectrophotometry by Dr. Harlow, are functional electron carriers in the ameba. It is presumed, but not proved, that there is a close molecular organization of the flavin moiety and the iron-sulfur centers of the electron-transferring proteins of E. histolytica.

Studies of the respiratory chain in Staphylococcus aureus were resumed in collaboration with Dr. Theodore, Laboratory of Microbiology, NIAID. Respiration of the protoplast membrane fraction of S. aureus, supported by lactate, glycerol-3-phosphate, and NADH, was inhibited by metal chelating agents. Inhibition was correlated with the capacity of the chelators to form coordination complexes with non-heme iron. These preliminary experiments indicate the presence and functional requirement for non-heme iron in the bacterial respiratory chain.

Mammalian studies: In conjunction with Dr. Tschudy, NCI, investigation of the interrelationship of heme synthesis and mitochondrial bioenergetics was renewed. Experimental hepatic porphyria was induced in rats with allylisopropylacetamide, and at various intervals after administration of the drug, liver mitochondria were isolated and assayed for the typical reactions of oxidative phosphorylation. No differences in State 3 respiration, respiratory control indices or P:O ratios were observed between the control and experimental groups. Rates of NADH oxidation were increased 150% in mitochondria from the experimental animals, a finding of considerable interest in view of previous work that porphyrin synthesis may be regulated by the cellular level of NADH/NAD+.

This investigation which attempts to elucidate vital biochemical mechanisms common to all aerobic cells, has obvious importance for biochemical research in general (as shown by numerous collaborative projects in which we have participated during past years), and provides a basis for understanding bioenergetic problems associated with parasites and parasitism.

The parasite studies will be continued with emphasis on further purification, characterization and elucidation of the physiological role of iron-sulfur flavoproteins in E. histolytica. Studies of L-serine metabolism in this parasite have been completed by Dr. Takeuchi, who returned to Japan in midyear. Mammalian work will be continued with primary emphasis on the renewed investigations with Dr. Tschudy on hepatic porphyria. It is anticipated that studies of diaphorase activity in human platelets, suspended during this year, will be renewed. Continued investigation of the respiratory chain of S. aureus will focus on the role of iron-sulfur proteins in electron-transfer reactions of the protoplast membrane.

Publication:

Takeuchi, T., Weinbach, E.C. and Diamond, L.S.: Pyruvate oxidase (Co A acetylating) in Entamoeba histolytica. Biochem. Biophys. Res. Commun. 65: 591-596, 1975.

von Brand, T. and Weinbach, E.C.: Incorporation of calcium into the soft tissues and calcareous corpuscles of larval Taenia taeniaeformis. Z. Parasitenkd. 48: 53-63, 1975.

Weinbach, E.C., Diamond, L.S., Claggett, C.E. and Kon, H.: Iron-sulfur proteins of Entamoeba histolytica. J. Parasitol. 62: 127-128, 1976.

Harlow, D.R., Weinbach, E.C. and Diamond, L.S.: Nicotinamide nucleotide transhydrogenase in Entamoeba histolytica, a protozoan lacking mitochondria. Comp. Biochem. Physiol. 53B: 141-144, 1976.

Weinbach, E.C., Harlow, D.R., Takeuchi, T., Diamond, L.S., Claggett, C.E. and Kon, H.: Aerobic metabolism of Entamoeba histolytica: Facts and fallacies. Arch. Invest. Med. (Mexico). (in press).

Weinbach, E.C., Harlow, D.R., Claggett, C.E. and Diamond, L.S.: Entamoeba histolytica: Diaphorase activities. Exp. Parasitol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00099-06 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (30 characters or less)

Biophysical Parasitology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dvorak, J. A. Research Microbiologist LPD NIAID
Other: None

COOPERATING UNITS (if any) Biomedical Engineering and Instrumentation Branch, DRS; Television Engineering Section, CC

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
24/12	12/12	12/12

SUMMARY OF WORK (200 words or less - underline keywords)

A multiparametric approach to a study of the penetration phase of Trypanosoma cruzi-vertebrate cell interactions is utilized in order to develop a mathematical model of the phenomenon. The effects of varying physiologic parameters will be studied in this system and compared with other host-parasite systems such as Toxoplasma gondii-vertebrate cell interactions. Low-light-level video techniques have been expanded to include clinical applications such as studies of eplieptiform siezures and myocardial infarction. Video microscopy techniques have been expanded to include the semiautomated analysis of microtiter plates.

Project Description:

The biophysical parasitology program has two major objectives: (1) To elucidate and attempt to understand the basic physical/chemical parameters responsible for obligate intracellular parasitism. More specifically, the program is restricted to studies of obligate intracellular protozoan parasites of medical importance. (2) To develop unique, primarily electro-optical, instrumentation to facilitate the study of obligate intracellular parasitism. Parallel studies are also in progress to apply the instrumentation developed with the program to other areas of biomedical research.

1. The interaction of Trypanosoma cruzi with vertebrate cells

A. A mathematical analysis of the penetration phase.

A multiparametric approach to the problem of penetration of vertebrate cells by T. cruzi trypomastigotes was developed which utilizes the controlled-environment culture system, some of the electro-optical instrumentation and new techniques for data reduction and analysis developed with the program. The object of the work is to produce a reliable mathematical model describing the penetration of vertebrate cells by T. cruzi trypomastigotes. Once this has been accomplished, the model should prove useful for study of this specific interaction and have general applicability to other host-parasite systems.

It was previously established that the distribution of a population of host cells is best described by a negative binomial equation. Recent studies have shown that this negative binomial distribution may reflect some form of differential susceptibility of the host cell population. The susceptibility of a host cell population is temporally unstable. Furthermore, it appears that the infection of a host cell by a parasite may influence subsequent invasions of the same host cell by parasites. This implies that although the early aspects of the interaction can be best described by the concept of differential susceptibility, there is also a contagious aspect to the interaction that subsequently appears.

During the penetration of bovine embryo skeletal muscle cells by trypomastigotes the concentration of extracellular parasites is a determinant of the incremental increase in the number of parasites penetrating the host cells in time. This concept is not as simple as it appears. Obviously, if there are no extracellular parasites, there will be no intracellular parasites either. However, if the host cell population attracts parasites but is resistant to penetration or if there is some threshold value for the total number of parasite "hits" of a host cell prior to penetration as defined by target theory, the linear interdependence of extracellular parasites and the incremental increase in intracellular parasites would not occur.

The onset of an infection of vertebrate cells by T. cruzi trypomastigotes is best described as a gamma distribution. This implies that there is a lag period prior to the onset of infection, followed by a period of peak penetration efficiency and a gradual decay in efficiency. Preliminary data infers that these phenomena may be related to changes in physical chemical parameters

of the medium.

B. Ultrastructural analysis of T. cruzi-vertebrate cell interactions. Two aspects of the interaction of T. cruzi with vertebrate cells are being studied. The first concerns the interiorization of the trypomastigote into the vertebrate cell. Although both the host cell and the parasite are membrane bound, an amastigote reproducing within a host cell is not enclosed in a host cell membrane-bound vesicle. There are three conceivable processes by which this phenomenon could occur. First, it could be an active process on the part of the parasite involving an actual puncture violation of the host cell membrane during penetration. Second, it could be a semiactive process on the part of the parasite where following the contact of a host cell membrane by a parasite there is a fusion of host cell and parasite membranes resulting in the interiorization of the parasite. Third, it could be a passive process on the part of the parasite. In this case the host cell would phagocytize the parasite and once the parasite is interiorized the phagocytotic vacuole enclosing the parasite would be broken down releasing the parasite inside the host cell. Techniques for studying this phenomenon have only recently been formalized and preliminary data support the hypothesis that host cell membrane is interiorized with the parasite. This membrane then appears to break down, in an irregular pattern, during the phase of reorganization of the parasite from a trypomastigote to an amastigote.

The second aspect of the interaction of T. cruzi with vertebrate cells being studied at the ultrastructural level involved the transformation of the kinetoplast during reorganization of a trypomastigote to an amastigote and, following the reproductive cycle from amastigote to trypomastigote. Kinetoplast transformations consist of changes in individual fiber diameter and geometry due to changes in the tertiary structure of the kinetoplast. These morphological changes correspond to transition points in the parasite's life cycle either from an extracellular to intracellular phase or vice versa and probably reflect differences in the physiologic requirements of the parasite.

II. The interaction of Toxoplasma gondii with vertebrate cells.

The penetration of a vertebrate cell by T. gondii as well as the subsequent first reproduction cycle of the parasite are influenced by the partial pressure of CO_2 disolved in the medium and the bicarbonate ion concentration of the medium. The ability of a population of parasites to penetrate a population of vertebrate cells is inversely proportional to the partial pressure of CO_2 in the medium. That is, increasing CO_2 concentrations are inhibitory. The bicarbonate ion concentration also influences penetration and this relationship is best described by a parabolic equation. Consequently, it appears that there may be at least two physiologic processes involved: CO_2 may be affecting parasite respiration, and bicarbonate ion may be acting as a substrate for some biosynthetic reaction involved in penetation.

III. Malaria Research

Close collaboration between this laboratory and the Malaria Section continues in an effort to define basic cullular aspects of malaria. Primary emphasis has shifted from visualization and recording of merozoite-erythrocyte interactions as the electro-optical system for this work has been formalized and is now routinely functional. Primary emphasis is now being directed toward the development of continuous in vitro culture systems (See report of L. H. Miller for details).

IV. Electro-optical instrumentation development

A. Semiautomated analysis of reactions performed in microtiter plates.

Several bioassay procedures such as Eosinophile Stimulation Promotor (BSP) reactions and Macrophage Inhibition Factor (MIF) reactions can be performed in microtiter plates. However, one of the major problems with the procedure is the time required for manual data collection and processing as well as the accuracy of the manually collected data. Instrumentation has been developed that permits the rapid collection and processing of microtiter plate data with a high degree of accuracy. An individual well of the microtiter plate is viewed through a microscope at low magnification. The resultant image is processes by a video integrator equipped with used definable delimiters. The resultant analog signal is digitalized and either processed in real time or stored on paper tape for subsequent analysis. A 96 well microtiter plate can be processed, including statistical analysis, in less than 30 minutes.

B. Low-light-level video instrumentation.

The low-light-level techniques described in previous reports are being applied to studies of electrical activity of the brain during splileptiform siezures and ischemic changes in the heart during myocardial infarction. Both of these procedures utilize probes involving the induced fluorescence of either fluorescene or the NADH-NAD transition. The techniques are non-invasive and non-destructive. Preliminary data correlate well with previous studies utilizing conventional approaches to the problems.

Significance and Proposed Course of Program:

(1) The study of host-parasite interactions at the cullular and subcellular level is a topic of critical importance. The outcome of this interaction directly influences the physiologic state and survival potential of both the host and the parasite.

(2) The methodology and research philosophy being developed are directly applicable to other areas of biomedical research. For example, the controlled-environment culture system developed with this program is now in routine use in laboratories with such diverse interests as oncology, immunology, neuro-physiology and morphogenesis. The video systems developed with this program are being used for analysis of macroscopic as well as microscopic images in basic research and diagnostic clinical-medical fields.

Research will continue in an attempt to further our understanding of the interaction of T. cruzi with vertebrate cells in vitro and relate this information to the course of Chagas' disease in nature. Basic information obtained with the controlled-environment culture system and video systems will be utilized in more complex in vitro model systems.

The basic methodology and research philosophy developed with this program will be used for the analysis of other host-parasite interactions. Most specifically, studies of the interaction between malaria merozoites and erythrocytes will be continued.

Publications:

Dvorak, J.A., Schuette, W.H., and Whitehouse, W.C.: The application of low light level video techniques to biomedical research. Optical Engineering (in press).

Fox, C.H., Dvorak, J.A., and Sanford, K.K.: Cytometric analysis of neoplastic transformation of vertebrate cell populations. Cancer Research (in press).

Miller, L.H., Shiroishi, T., and Dvorak, J.A.: Enzumatic modification of the erythrocyte membrane surface and its effect on malaria merozoite invasion. J. Mol. Med. 1: 55-63, 1975.

Miller, L.H., Aikawa, M., and Dvorak, J.A.: Malaria (Plasmodium knowlesi) merozoites: Immunity and the surface coat. J. Immunol. 114: 1237-1242, 1975.

Dvorak, J.A.: A new in vitro approach to quantitative studies of Trypanosoma cruzi. Proceeding of the International Symposium on New Approaches in American Trypanosomiasis (in press).

Miller, L.H., Mason, S.J., Dvorak, J.A., McGinniss, M.H., and Rothman, I.K.: Erythrocyte receptors for (Plasmodium knowlesi) malaria: The Duffy blood group determinants. Science 189: 561-563, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00100-04 LPD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Terminal Electron Transport in Parasites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.R. Harlow	Senior Staff Fellow	LPD NIAID
Other:	E.C. Weinbach	Head, Physiology & Biochemistry	LPD NIAID
	L.S. Diamond	Head, Parasite Growth & Differential	LPD NIAID
	S.J. Mason	Research Associate	LPD NIAID
	L.H. Miller	Head, Malaria Section	LPD NIAID

COOPERATING UNITS (if any)

Dr. Carl Phillips, Finnigan Corporation, Sunnyvale, California

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Section on Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12/12	12/12	0

SUMMARY OF WORK (200 words or less - underline keywords)

The primary objective of this project is the characterization of electron transport systems of parasites. Current areas of concentrated effort are electron transport systems of malarial parasites and Entamoeba histolytica. Major compounds of interest are cytochromes and iron-sulfur proteins. The presence of cytochromes are detected by optical spectrophotometry, especially low-temperature spectrophotometry. Iron-sulfur proteins are detected by electron paramagnetic resonance.

The importance of iron-sulfur proteins to electron transport in E. histolytica suggests a nutritional requirement for iron and sulfur in the amoeba. We are attempting to improve axenic growth of the amoeba in less complex culture media by the addition of iron and sulfur containing compounds. In addition, studies are done on improving in vivo infectivity of the amoeba in laboratory animals by concurrent administration of iron-containing compounds.

Project Description:

(1) Study of heme pigments in the electron transport systems of malarial parasites has been impeded in the past by the very large quantities of heme-iron present in the hemolgobin of the host red blood cell. This hemoglobin heme-iron "masks" the other heme-iron pigments such as cytochromes when spectrophotometric analyses are performed on the parasite-host cell system. Drs. Mason and Miller have perfected a procedure for isolating the malarial parasites substantially free from the red blood cell hemoglobin. Spectrophotometric studies of these isolated malarial parasites have shown differences between pigments in Plasmodium knowlesi and Plasmodium inui. P. inui, a parasite lacking mitochondria, shows a much weaker absorbtion centered at 638 nm. Further evidence of different electron transport compounds in these two species are EPR spectra which show a large signal in the rubredoxin region for P. knowlesi which is totally lacking in P. inui.

The final stage of the spectrophotometric analyses (low-temperature spectrometry) has been delayed by breakage of a component used in these analyses. Upon receipt of the replacement, this phase will be completed. The EPR spectra raise the possibility that rubredoxin-like iron-sulfur proteins are components of the electron transport chain of P. knowlesi (no mitochondria) but are lacking in P. inui (mitochondria present). Further studies may indicate similarities between the electron transport systems of P. knowlesi and Entamoeba histolytica which also lacks mitochondria.

(2) Investigations in collaboration with Dr. Weinbach on the electron transport iron-sulfur proteins of Entamoeba histolytica have extended our knowledge the amoeba's electron transport chain. Quantitative reduction of the iron-sulfur center in diaphorase with NADPH resulted in proportional increases in the size of the EPR signal indicating a physiological function for the iron-sulfur center (i.e. the oxidation of NADPH). Modification of the EPR signal when the amoebae are grown in a ^{57}Fe-enriched medium proves the participation of iron in the formation of the distinctive EPR signal. These studies, together with Dr. Weinbach's previous chemical analyses of non-heme iron and sulfur in the amoeba, leave little doubt that iron-sulfur proteins are important components in E. histolytica electron transport.

(3) The importance of iron-sulfur proteins in the electron transport system of E. histolytica stimulated co-operative work with Dr. Diamond on improving the axenic growth of the amoeba on less complex media supplemented with iron and sulfur. Energy dispersive x-ray spectrometry of the amoebae and various culture media (in California) provided the following data: (1) the amoeba concentrate iron from the culture media, (2) the complex media, TPS-1 contained much more iron than did the less complex media, Biosate. These findings explained the enhancement of E. histolytica growth when iron compounds are added to Biosate media. Such iron additions to the complex media, TPS-1, showed no enhancement indicating the presence of sufficient iron in this medium. It is known that amoeba require addition of sulfur-containing compounds, especially cysteine, for maximal axenic growth. This requirement could be related to the sulfur needed in the synthesis of the iron-sulfur proteins.

Attempts at acclimatizing the amoebae to grow in defined media supplemented with iron, sulfur-containing amino acids, and other constitutents are now under way.

(4) Also in co-operation with Dr. Diamond's group, studies have been initiated on the effect of iron compounds on in vivo infections of Entamoeba histolytica in laboratory animals. The results of initial studies suggest a stimulatory effect on infection by concurrent administration of iron-containing compounds. Future work will amplify and clarify this effect.

Significance to Bio-medical Research and the Program of the Institute:

Parasites characteristically exhibit an anaerobic metabolism. This generally accepted dogma is confused by the fact that many parasites consume oxygen when it is available. Resolution of these conflicting observations has not been possible since the terminal electron transport mechanisms of parasites (e.g. presence or absence of mammalian-type cytochromes) are not known. It is of primary importance to determine the means by which parasites perform these vital biological oxidation-reductions both from the aspect of possible chemotherapeutic value, and from the aspect of intrinsic scientific merit.

Publications:

Harlow, D.R., Weinbach, E.C. and Diamond, L.S. Nicotinamide nucleotide transhydrogenase in Entamoeba histolytica, a protozoan lacking mitrochondria. Comp. Biochem. Physiol. 53B: 141-144, 1976.

Weinbach, E.C., Harlow, D.R., Claggett, C.E. and Diamond, L.S. Entamoeba histolytica: Diaphorase activities. Exp. Parasitol. (In press).

Weinbach, E.C., Harlow, D.R., Takeuchi, T., Diamond, L.S., Claggett, C.E. and Kon, H. Aerobic metabolism of Entamoeba histolytica: Facts and fallacies. Arch. Invest. Med. (Mexico) (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00101-02 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization of Polysaccharides in Protozoa of the family Trypanosomatidae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Gottlieb, M. Staff Fellow LPD NIAID
Other: Wieder, S. Laboratory Worker LPD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13/12	12/12	1/12

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to characterize the polysaccharides isolated from certain species of parasitic protozoan hemoflagellates of the family Trypanosomatidae, including the human pathogen Trypanosoma cruzi. Topics under present and future investigation include; 1) the chemical nature of the polysaccharide and its possible linkage to other molecules; 2) subcellular localization of these molecules; 3) the antigenic and immunological properties of these molecules and 4) possible relationship of these molecules to the stage of the parasite's life cycle. The characterization of the isolated carbohydrate indicates nonglycogen-like neutral polysaccharides in addition to negatively charged molecules. The antigenic nature of the polysaccharide has been shown by immunodiffusion and complement fixation techniques.

Project Description:

Flagellate protozoa of the family Trypanosomatidae do not store carbohydrate as an energy reserve. However, substantial quantities of polysaccharide are present in these organisms. Our interest on characterizing this material has focused on its possible role in membrane structure and on its immunological properties.

Carbohydrate has been isolated from Crithidia fasciculata, a parasite of insects, and the culture (epimastigote) form of Trypanosoma cruzi by chemical fractionation techniques including phenol and chloroform-n-amyl alcohol extraction procedures. Further isolation and chemical properties of the polysaccharide molecules have been investigated using standard methods; e.g., chromatography, enzyme digestion and radiolabeling.

Biochemical Characterization:

C. fasciculata: The crude polysaccharide isolated from this species has been fractionated by ion exchange and molecular sieve chromatography into two components. One fraction, a neutral polysaccharide; is composed solely of mannose; it is heterodisperse with an average molecular weight of 14,000. The other fraction is bound to DEAE cellulose even in the presence of high salt but may be eluted with detergent (suggesting possible strong non-ionic interactions). This polysaccharide is tentatively identified as containing the neutral sugars arabinose and galactose and has a probable molecular weight greater than 200,000. Electrophoresis of the crude polysaccharide yields one very broad, periodic acid Schiff (PAS) positive band which migrates to the anode. (The purified mannan does not migrate in an electric field).

Fractionation of whole cells by differential centrifugation indicates that most of the polysaccharide is localized within the particulate fraction of the cell; less than 20% of the total is extracted from the soluble portion.

T. cruzi: There is less polysaccharide per weight of this species than per weight of C. fasciculata. However, the crude polysaccharide apparently is more complex; i.e., in the number of molecules present in the crude preparation and in physical properties. The extracted polysaccharide readily forms micelles and aggregates which makes handling difficult. As with C. fasciculata preparations, much of the T. cruzi polysaccharide remains bound to DEAE cellulose even at high salt concentrations and is eluted by detergent. Analysis of the neutral sugar fraction shows the sample to be composed of galactose and mannose.

Samples electrophoresed on polyacrylamide gels yield upon PAS staining, a profile revealing at least three discrete bands; one of these may be a glycoprotein.

The plant lectin concanavalin A agglutinates whole T. cruzi cells and precipitates a fraction from the polysaccharide preparasiton from this organism. Neither of these activities is seen with C. fasciculata.

Antibody Studies:

Precipitating antibodies to the crude polysaccharide are present in hyperimmune sera from rabbits immunized with either homogenates of whole cells or the crude polysaccharide itself. Antibodies to T. cruzi polysaccharide (detected by immunodiffusion or complement fixation) have not been demonstrated in a serum of an individual with known infection and disease and only to a very low titer in a pool of human sera known to respond to other T. cruzi antigens.

Evidence is adduced, primarily from histochemistry, that polysaccharide is a component of the trypanosomatid membrane primarily the plasma membrane. Therefore, a further knowledge of the polysaccharide chemistry may help us to understand the nature of the surface and possible recognition sites of these parasites. We are examining interactions of antibodies and lectins to the cell surface and hope to isolate surface and membrane components to establish identity with the extracted polysaccharides. Further work will examine the changes which may occur with respect to the surface and polysaccharide material during the course of the life cycle of T. cruzi.

While these studies on the polysaccharides have been carried out, work on aspects of protein synthesis in these organisms has been suspended.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00102-02 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Neva	Chief, Laboratory of Parasitic Diseases	LPD NIAID
Other:	J. Weintraub	Research Associate	LPD NIAID
	A. Gam	Biological Laboratory Technician (micro)	LPD NIAID

COOPERATING UNITS (if any)

Dr. Fred Weinbaum, Research Associate, LMI, NIAID
Walter Reed Army Institute of Research

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
39/12	17/12	22/12

SUMMARY OF WORK (200 words or less - underline keywords)

A model of leishmanial infection (L. tropica) in the foot pad of in-bred mice has been found to be reproducible and useful for immunological and chemotherapeutic studies. Metastatic spread of the infection could not be attributed to impairment of the immune system as assessed by hemolytic plaque formation, and was not enhanced by an anti-B cell serum. The availability of different strains of leishmania that produce a spectrum of responses in the mouse model and cryopreservation techniques for maintenance of strains will permit comparative studies with this system.

The amastigote-trypomastigote antigen prepared from cell cultures infected with T. cruzi was studied by gel diffusion procedures. The results by counter-immuno electrophoresis parallel very closely those obtained in complement-fixation tests. In those instances where specificity of reactions is in doubt, sera are examined by a variety of tests including IFA. Cell cultures are also being used to assess the infectivity of different strains of T. cruzi. These tests can be applied to organism directly from infected triatomine bugs.

Project Description:

Further experience has confirmed the reproducibility and usefulness of the leishmaniasis model (L. tropica) in the foot pad of in-bred mice. Spread of infection in this system could not be attributed to failure of response of the immune system. The splenic plaque-forming cell response was actually enhanced in infected animals during a period when organisms disseminate and was depressed only in late stages of infection. Animals whose B cell activity was deliberately supressed by an anti-B cell serum exhibited no obvious change in the course of their infection. Several experimental anti-malarial drugs which gave promising results vs. L. donovani in hamsters showed very poor activity in the L. tropica mouse model, but earlier BCG infection clearly inhibited the leishmanial lesion.

We now have several additional strains of leishmanial organisms from human cases of cutaneous as well as visceral disease which exhibit a wide spectrum of responses in the in-bred mouse. Since the organisms can be frozen in liquid nitrogen with 50% viability or they can be studied in a comparative fashion without the danger of altering characteristics by passage. Another strain of L. tropica, for example, produces a barely detectible lesion in the mouse foot-pad but can be recovered from the injection site up to six months later. A Central American strain of L. braziliensis, in contrast, consistently produces a modest lesion in the foot pad and then resolves spontaneously.

Work with the Trypanosoma cruzi antigen from infected cell cultures focused largely upon analysis of its reactivity in gel diffusion systems. All human sera known to be positive reactors to the cell culture antigen in complement-fixation (CF) tests were also positive by counter-current immuno-electrophoresis (CIE) with the same antigen. A false positive CIE test was observed with the T. cruzi cell culture antigen and serum from a case of Kala-azar. Twenty-nine individuals from Texas who had participated in a 1969 nutrition survey and found to have questionably positive CF reactions to T. cruzi were located, examined and bled for re-testing. Only 7 of these follow-up sera gave positive CF reactions, all at low dilutions, and 7 additional specimens gave equivocally positive reactions. Although these serum samples will also be checked by the IFA test, it is still not entirely clear whether they represent individuals who acquired infection with T. cruzi in this country.

A new approach was made to assessing infectivity of T. cruzi for cells in vitro by formation of plaques under agar. In addition, these findings have been correlated with results of infectivity of inocula by terminal dilution and trypomastigote production in two-step growth curves. These tests can be applied directly to organisms from infected bugs and have also been made at high (38°C) and low (33°) temperatures. The growth curves are easy to perform, and the terminal dilution and plaquing procedures appear to yield promising results in preliminary tests. These procedures would be useful for more precise comparison of different strains of T. cruzi and as a tool for genetic studies with this organism.

Publications:

Raizman, R.E. and Neva, F.A.: Detection of circulating antigen in acute experimental infections with Toxoplasma gondii. J. Infect. Dis. 132: 44-48, 1975.

Sheffield, H.G., Melton, M.L. and Neva, F.A.: Development of Hammondia hammondi in cell cultures. Proceedings of the Helminthological Society of Washington. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00103-09 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunological Studies on Toxoplasmosis and Other Parasitic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Milford N. Lunde	Research Zoologist	LPD NIAID
OTHER:	Louis S. Diamond	Head, Parasite Growth and Differentiation Section	LPD NIAID
	Ronald Fayer	Research Parasitologist	Agricultural Research Service

COOPERATING UNITS (if any)

Animal Parasitology Institute, Agricultural Research Service Service, Beltsville, Maryland

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasite Growth and Differentiation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	13/12	5/12

SUMMARY OF WORK (200 words or less - underline keywords)

In the search for improved sero-diagnostic tests, we have eliminated non-specific precipitins in E. histolitica antigen used in counter current immunoelectrophoresis with the barbital-borate buffer system. This was done by passing the antigen through a modified calcium phosphate column. Antigen which was only slightly reactive in the hemagglutination test was active in counter current electrophoresis. Soluble antigen has been prepared from Sarcocystis fusiformos zoites and has been successfully used to detect antibody to Sarcocystis in experimentally infected cattle.

PHS-6040
(12-75)

Project Description:

The search for improved sero-diagnostic tests and attempts to better understand the immune factors involved in host parasite relationship require antigens which are specific and at the same time have a high degree of sensitivity.

Purification of E. histolytica antigen was obtained using an immunoadsorbent column to which globulin from a patient with an amebic liver abcess had been bound. Antigen was eluded from the column with 5 M Sodium Iodide. Since the efficiency of this procedure was low, only about 10% of the antigen added to the column being recovered, another more efficient method of fractionating E. histolytica antigen had to be found. A modified calcium phosphate (hydroxylapatite) column appeared to give good separation of antigenically active and inactive components of E. histolytica. It was found that unfractionated antigen gave a non-specific precipitin arc with most normal human sera when the counter current immunoelectrophoresis (CIEP) procedure was used employing a barbital-borate buffer. This has been removed by passing the antigen through the hydroxylapatite column with the barbital borate buffer pH 8.6, ionic strength 0.1 M (Fraction-1) followed by elution with 0.45 M sodium phosphate pH 6.8 (Fraction-2). Both Fraction 1 and Fraction 2 gave E. histolytica positive precipitins by CIEP with sera from a patient with amebic liver abcess while with negative control sera no non-specific precipitin lines or artifact were observed. Most of the hemagglutination antigen was, however, found in Fraction 2.

By the use of fractionated antigens in sensitized rats and/or guinea pigs we are attempting to identify the reagin responsible for skin sensitivity which ultimately may result in production of a more specific skin test antigen. We also wish to investigate any possible relationship between tissue sensitivity and intestinal or hepatic amebic lesions.

Studies started last year to determine the presence of IgA and IgM antibodies intestinal and hepatic amebiasis have been discontinued due to lack of sufficient clinical material.

Soluble antigen has been prepared from Sarcocystis fusiformis zoites which were obtained by enzyme digestion from bovine myocardium. This antigen has been successfully used in an indirect hemagglutination (IHA) and agar gel diffusion tests to detect antibody to Sarcocystis in experimentally infected cattle. Although Sarcocystis is closely related to Toxoplasma, antibody to which is also found in cattle, no evidence of cross reactivity between these two parasites has been found.

Since Sarcocystis has been found in beef which constitutes a large part of the American diet and human infection with this parasite has been reported in both Europe and Asia, a means of detecting antibody to it is needed.

Studies on the role of serum soluble antigens and antigen-antibody complexes in toxoplasmosis are also in progress.

Publications:

Sheagren, J.N., Lunde, M.N. and Simon, H.B.: Chronic lymphadenopathic toxoplasmosis: A case with marked hyperglobulinemia and impaired delayed hypersensitivity responses during active infection. Amer. J. Med. 60: 300-305, 1976.

Lunde, M.N., and Powers, K.G.: The preparation of malaria haemagglutination antigen. Ann. Trop. Med. and Parasitol. 70(3): (in press).

Fayer, R., Johnson, A.J. and Lunde, M.N.: Abortion and other signs of disease in cows following experimental infection with sarcocystis from dogs. J. Infect. Dis. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT ...	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00104-02 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure and Development of Parasitic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H.G. Sheffield Head, Section of Cell Biology and Immunology LPD NIAID
Other: M. L. Melton Research Zoologist LPD NIAID

COOPERATING UNITS (if any)

Animal Parasitology Institute, ARS-USDA, Beltsville, Maryland
Department of Pathology and Oncology, School of Medicine, Uinversity of Kansas, Kansas City, Kansas

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Section on Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
30/12	12/12	18/12

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the cell biology of various parasites in relation to the organism itself, its interaction with the host cell and production of disease. Current areas of interest are: 1) development of immunity; 2) effect of drugs on the parasite life cycle; 3) comparative ultra structure of infective forms (sporozoites) of related parasites; and 4) asexual and sexual development of the parasite. Emphasis is placed on Toxoplasma gondii and toxoplasmosis with members of the genera Hammondia, Sarcocystis and Isospora being used for comparative purposes. Techniques used include electron microscopy, tissue culture, serology and animal infections.

PHS-6040

Project Description:

Cellular structure and changes related to growth, differentiation and multiplication of parasitic protozoa and response of host tissue to parasitic infection are studied. Aspects of infection and disease related to structure and development, particularly in toxoplasmosis, are examined.

Immunological response in relation to prevention of reinfection is studied in toxoplasma-infected cats. In addition to standard parasitological techniques, in vitro cultivation and electron microscopy are utilized.

Attempts to immunize cats against oocyst-producing infections of Toxoplasma gondii have been continued. Lasalocid, an anticoccidial drug, was used prophylactically in cats at a level of 10 mg/kg body weight per day. This level, twice that used previously, did not prevent oocyst excretion but did reduce the number of oocyst produced and lengthened the prepatent period. Both control and treated cats were immune to an oocyst-producing reinfection when challenged at 54 and 112 days after infection.

Of seven cats infected with C-56 strain T. gondii and challenged two months later, only one produced oocysts when challenged a second time with the same strain of organisms eleven months after the first challenge. Six of these cats, including the positive one, were challenged a third time two months after the second challenge. Theee of them were challenged with the Sterling strain and three with the M-7741 strain. None produced oocysts indicating that immunity is not strain specific.

In a preliminary study of susceptibility of cats to different stages of T. gondii, three cats were inoculated with either artificially excysted sporozoites, tachyzoites harvested from the media of cell cultures, or bradyzoites of the small intestine approximately 12 cm from the pylorus after laparotomy. Oocysts were produced on days 6-11 and 7-15 in bradyzoite and tachyzoite-infected cats, respectively. Sporozoite-injection did not result in oocyst production nor in development of test antibody. However, sporozoite infectivity was demonstrated in cell cultures.

The effectiveness of sulfisoxazole, a highly soluble sulfonamide which readily passes the blood-brain barrier, was compared with sulfadiazine against T. gondii in cell cultures. Destruction of cell layer by the parasites was reduced by 5 ug/ml of sulfadiazine whereas 50 ug/ml of sulfisoxazole was required to produce the same effect. This data confirms in vivo results which indicated that sulfadiazine is six to ten times as effective as sulfisoxazole.

Ultrastructural studies of the development of Sarcocystis fusiformis in the intestinal tissues of dogs have been continued. The earliest stages found were seen three days after feeding infected cardiac tissue to the dogs. These stages, apparently gametocytes, were uninucleate, had a thin wall, and had minimal cytoplasmic structure. It is believed that these forms result from invasion of intestinal edothelium by zoites releaded from cysts in bovine

cardiac muscle following digestion. No schizogonic stages were observed. Lack of asexual multiplication in the final host is a characteristic separating Sarcocystis from Toxoplasma and other coccidia.

Study of the ultrastructure of sporozoites of Toxoplasma, Sarcocystis, Hammondia and Isospora has been completed. Their close taxonomic relationship is confirmed by their common structural characteristics. All have a two layer pellicle with an anterior complex including a conoid, micronemes and rhopries which are assumed to facilitite cell penetration. A significant difference between Toxoplasma and the others is the presence, in the latter, of small storage granules which often have a crystalline appearance. Studies of H. hammondi in cultured cells have shown that these storage granules disappear following cell invasion.

Studies on development of the cyst and bradyzoites of Sarcocystis has been initiated using S. muris in the mouse as a model system. Early stage cysts contain dividing forms called metrocytes. After an undetermined number of divisions bradyzoites are formed and accumulate in the center of the cyst while metrocytes are still present around the periphery. Old cysts contain only bradyzoites. Cysts are not infective for cats, the final host, until bradyzoites are formed. Little evidence of muscle cell degeneration was seen adjacent to viable cysts. Study of the cyst wall and the host-parasite relationships involved is in progress.

Toxoplasma gondii is an unusual organism because of its ability to invade and multiply within almost any mammalian cell type. In humans it is neurotropic and can cause serious disease. Although many aspects of the parasite and the disease it produces have been studied several important points in the area of cell biology need to be clarified: 1) How does the parasite enter the host cell? 2) What triggers rapidly dividing forms to slow down and begin cyst formation? 3) How does the parasite avoid being destroyed by the host cell? and 4) What requirements are necessary for the parasite to proceed through schizogonic and gametogonic stages rather than the tachyzoite-bradyzoite cycle seen in cells other than cat intestinal epithelium? Further studies on Toxoplasma gondii and related organisms such as Hammondia hammondi, Sarcocystis spp and other coccidia utilizing morphological, cyto-chemical and immunological techniques are planned to help answer these questions.

Publications:

Sheffield, H.G. and Melton, M.L.: Effect of pyrimethamine and sulfadiazine on the fine structure and multiplication of Toxoplasma gondii in cell cultures. J. Parasitol. 61: 704-712, 1975.

Melton, M.L. and Sheffield, H.G.: Activity of the anticoccidial compound, lasalocid, against Toxoplasma gondii in cultured cells, J. Parasitol. 61: 713-717, 1975.

Sheffield, H.G. and Melton, M.L.: Effects of pyrimethamine and sulfadiazine

on the intestinal development of Toxoplasma gondii in cats. Am. J. Trop. Med. Hyg. (in press) 1976.

Sheffield, H.G., Melton, M.L., and Neva, F.A.: Development of Hammondia hammondi in cell cultures. Proc. Helm. Soc. Wash. (in press) 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00105-04 5 LPD

PERIOD COVERED
July 1, 1975 to ~~June~~ September 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanism of Invasion of Erythrocytes by Malaria Merozoites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. H. Miller	Head, Malaria Section	LPD NIAID
Other:	S. J. Mason	Research Associate	LPD NIAID
	J. A. Dvorak	Research Microbiologist	LPD NIAID
	T. Shiroishi	Zoologist	LPD NIAID
	S. K. Martin	Visiting Fellow	LPD NIAID
	F. McAuliffe	Staff Fellow	LPD NIAID
	M. McGinniss	Research Biologist	BB CC
	M. Frank	Senior Scientist	LCI NIAID

COOPERATING UNITS (if any)

D. J. Haynes, Dept. of Immunology, Walter Reed Army Inst. of Research
C. Alving, Dept. of Immunology, WRAIR
M. Haut, Dept. of Hematology, WRAIR

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
~~4.7/12~~ 4.5	~~4.7/12~~ 4.5	

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to understand the merozoite and erythrocyte factors in the eventual interiorization of the merozoite within the erythrocyte. The research at present is to determine: (1) the receptor system on erythrocytes for initiating invasion of Plasmodium knowlesi, P. vivax and P. falciparum (2) the role of erythrocyte metabolism in parasite invasion and development; and (3) the merozoite structure in the surface coat that attaches to the receptors.

PHS-6040

Project Description:

Objectives:

1. Study of the P. knowlesi - P. vivax receptor system on erythrocytes.
   a. Are the Duffy blood determinants (Fy a+ and/or Fy b+) receptors for P. knowlesi?
   b. Is the Duffy negative genotype (FyFy) the basis for refractoriness to P. vivax?
   c. What is the chemical basis for the Duffy blood group system?

2. Study of the P. falciparum receptor system on erythrocytes.

Methods Employed:

1. Culture of P. knowlesi in Medium 199. Schizont-infected erythrocytes are mixed with test erythrocytes, and the suspension is added to limbro plates and cultured for three hours at 35°C in 2% CO_2. Smears from the plates are stained with Giemsa; the number of test erythrocytes invaded are counted.

2. Blood group phenotypes were determined by routine blood bank methods. Duffy typing of animal erythrocytes and enzyme-treated erythrocytes was performed by absorption of anti-Duffy a and anti-Duffy b antisera, followed by titering of the antisera.

3. F. 2 and II B glycolipid fractions of erythrocytes of known Duffy type were incorporated into liposomes and tested for Duffy antigenicity.

4. Anti-Duffy antibody was labelled by the lactoperoxidase, chloramine T, iodine monochloride and the Bolton-Hunter methods. The antibody was purified by absorption-elution.

5. Culture of P. falciparum. When the parasitemia in a chimpanzee was approximately 60%, erythrocytes were frozen in glycerol. At the time of culture, the infected erythrocytes were thawed and mixed with fifty times as many test erythrocytes. The invasion rate was analyzed on Giesma-stained smears.

Major Findings:

1. Evidence for the Duffy blood group antigens as receptors for P. knowlesi.
   a. A mutation from Duffy a to Duffy negative occurred in a family of Cree Indians of Northern Alberta, Canada. Since these erythrocytes were refractory to invasion by P. knowlesi (as was originally observed for Duffy negative erythrocytes of blacks), the Duffy allele and not some other closely linked gene determines P. knowlesi refractoriness.

b. Chymotrypsin treatment of human erythrocytes removed Duffy antigens and blocked invasion. Chymotrypsin treatment of rhesus erythrocytes did not block invasion and the Duffy determinants were not removed.
c. Erythrocytes that were ABO null, Rh null, Kell negative, Kx negative, P negative, Lu (a- b-) were all invaded.

2. Evidence that P. knowlesi merozoites can invade erythrocytes without Duffy a or b determinants.
a. Duffy negative human erythrocytes were susceptible to invasion after trypsin treatment. These treated erythrocytes were still Duffy negative.
b. New World monkeys that were susceptible to invasion in vitro and in vivo have no Duffy a or b determinants by methods used to date.

3. The Duffy negative genotype is the basis for refractoriness of blacks to P. vivax. We tested the blood group phenotype of prisoner volunteers who had previously been exposed to vivax-infected mosquitoes. Of the eleven blacks, six were Duffy blood group positive, Fy (a+ or b+), and were infected. The five with the Duffy negative genotype (FyFy) were all resistant to infection.

4. Chemical studies of Duffy determinants met with problems. ^{125}I labelling of the tyrosine groups on antibody inactivated the antibody. Labelling by the Bolton-Hunter method successfully labelled the antibody.

Erythrocyte glycolipids incorporated into liposomes had no Duffy acitvity.

5. The P. falciparum receptor on erythrocytes is not the Duffy Determinant.

Proposed Course:

1. Study the chemistry of Duffy blood group determinants.

2. Further testing of the vivax-Duffy hypothesis.

3. Determine the receptor for P. falciparum and its chemistry.

4. Determine innate resistance factors of erythrocytes. This will include biochemical studies of erythrocytes from children of severe malaria as compared to the general population. We also plan to study development of P. falciparum in enzyme deficient erythrocytes.

Significance:

We hope that identification of the receptor system will lead to techniques for interrupting the erythrocytic cycle. This is part of the institute's goal of malaria control through basic research.

Publications:

Aikawa, M., Miller, L.H. and Rabbege, J.: Erythrocyte membrane alteration in Plasmodium vivax and Plasmodium cynomolgi by electron microscopy and its possible relation to Schüffner's dots. Am. J. Pathol. 79: 285-300, 1975.

Miller, L.H., Aikawa, M., and Dvorak, J.A.: Malaria (Plasmodium knowlesi) merozoites: Immunity and the surface coat. J. Immunol. 114: 1237-1242, 1975.

Miller, L.H., Mason, S.J., Dvorak, J.A., Rothman, I, and McGinniss, M.: Erythrocyte receptors for (P. knowlesi) malaria: The Duffy blood group determinants. Science 189: 561-563, 1975.

Miller, L.H.: Method of malaria in Current Contents, 1975, pp. 36-38.

Miller, L.H., Shiroishi, T., Dvorak, J.A., Durocher, J. and Schrier, B.: Enzymatic modification of the erythrocyte membrane surface and its effect on malarial merozoite invasion. J. Mol. Med. 1: 55-63, 1975.

Miller, L.H., and Carter, R.: Innate resistance in malaria. Exp. Parasitol. (in press).

Miller, L.H., Powers, K.G., and Shiroishi, T.: Protective immunity and anti-merozoite antibodies. Exper. Parasitol. (in press).

Aikawa, M. and Miller, L.H.: Ultrastructural changes in the erythrocyte plasma membrane in ovale-type malarial parasites. J. Parasitol. (in press).

Miller, L.H., Mason, S.J., Clyde, D.F., McGinniss, M.H.: The resistance factor in blacks to Plasmodium vivax: The Duffy blood group genotype, FyFy. N. Engl. J. Med. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00107-05 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chemotherapy of Malaria and Other Protozoan Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard L. Jacobs	Research Biochemist	LPD NIAID
	Kendall G. Powers	Research Zoologist	LPD NIAID
Other:	Louis C. Koontz	Biological Laboratory Technician	LPD NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
15/12	6/12	9/12

SUMMARY OF WORK (200 words or less - underline keywords)

Both principal investigators left the NIH during the year. The time was spent writing up the work. The project has been discontinued.

Publications:

Jacobs, R.L. and Koontz, L.C.: Plasmodium berghei: Development of Resistance to Clindamycin and Minocycline in Mice. Experimental Parasitology. (in press)

Powers, K.G., Aikawa, M. and Nugent, K.M.: P. knowlesi: Morphology and Course of Infection in Rhesus Monkeys Treated with Clindamycin and its N-demethyl-4'-pentyl analogue. Experimental Parasitology. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00108-05

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Biology and Immunogenicity of Malarial Sporozoites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.H. Chen	Staff Fellow	LPD NIAID
Other:	R.W. Gwadz	Research Entomologist	LPD NIAID
	F.I. Weinbaum	Research Associate	LMI NIAID

COOPERATING UNITS (if any)

Laboratory of Microbial Immunity, NIAID

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
38/12	12/12	26/12

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the invasive and immune mechanisms involved in sporozoite-induced infections in rodent (Plasmodium berghei) and simian (Plasmodium knowlesi) malaria.

1) Techniques are being developed to follow the course of sporozoite invasion in vitro and in vivo.

2) Cellular requirements for generating protection using T-and B-cell deficient mouse models have demonstrated the absolute dependency of T-cells in the protective immune responses against sporozoite infections.

3) Limited success was obtained in the immunization of Rhesus monkeys against P. knowlesi sporozoites.

Project Description:

Significant findings.

In an attempt to clarify the mechanism of sporozoite-induced immunity using the P. berghei-rodent immunization model where T-deficient (ATX-BM-ATS) and B-deficient (μ-suppressed) mice were employed as hosts, significant findings are as follows:

1) B-cells alone are insufficient in generating protection; T-cells are absolutely required.

2) In the majority of cases T-cells can confer protection in the absence of B-cells.

3) B-cells may participate in a secondary role in confering complete protection in mice.

The biology and immunology of sporozoite-induced infections in rodent and simian malaria models are being studied, with emphasis on problems relating to sporozoite invasive mechanisms, immunogenicity, and cellular requirements leading to the development of resistance.

I. Rodent Malaria

A. Transmission. An improved cyclical transmission model of Plasmodium berghei has been established, and adequate number of sporozoites are being produced on a routine basis for experimental purposes.

B. Sporozoite Invasion. Optimal conditions for the direct viewing of sporozoite invasion in vitro have not been determined. In spite of the fact that exoerythrocytic stages are found in liver parenchymal cells, the sequence of events or pathways leading to the formation of these e.e. stages have not been established. Attempts were made to prepare highly-purified, host material freed, ^{3}H-adenine labelled sporozoites as a specific radioactive marker for sporozoite invasion in vivo. The degree of labelling per parasite from such preparations remains relatively low rendering auto-radiographic and liquid scintillation analyses of these parasites in tissues and organs impractical at this time.

C. Immunogenicity of Inactivated Sporozoites. Sporozoites inactivated by repeated freeze-thawing, heat treatment at various temperatures, or by addition of different concentrations of formaldehyde failed to protect mice against a sporozoite challenge, whereas the injection of X-irradiated sporozoites I.V. consistently produced animals resistant to challenge.

D. Mechanism of Sporozoite-Induced Immunity. The mechanism of sporozoite-induced immunity was examined by immunizing T-cell deficient (ATX-BM-ATS) and B-cell deficient (μ-suppressed) mice. All sporozoite immunized, T-cell deficient (adult thymectomized, X-irradiated, bone marrow reconstituted and anti-thymocyte serum treated) animals failed to resist a

sporozoite challenge. Their sera failed to abolish sporozoite infectivity after pretreatment, but sera from some of these immunized T-cell dificient mice did produce circumsporozoite precipitin (CSP) antibodies. The majority of sporozoite immunized, B-cell deficient (injected from birth with goat anti-mouse μ-chain globulins) animals were protected against a sporozoite challenge. Their sera had neither sporozoite neutralizing activity nor CSP antibodies.

These studies provided strong evidence that the requirement for T-cells in protection is absolute and more significantly that protection can be achieved without the aid of B-cells even though the latter is likely to contribute some role in augmenting immune responses to confer complete protection.

II. Simian Malaria

A. Immunization of Rhesus monkeys. Immunization of a limited number of Rhesus monkeys with X-irradiated P. knowlesi sporozoites generally produced negative results. Of 4 animals receiving attenuated sporozoites by I.V. injections, 3 were not protected after periods of immunization ranging from 2 weeks to 3 months (2 animals). The short -term immunized animal received a total of 30 X 10^6 sporozoites (3 injections) and the latter animals each received a total of 50 X 10^6 (6 injections) and 70 X 10^6 (8 injections) sporozoites. The 4th animal was resistant to sporozoite challenge after a vaccination period of 5.5 months after receiving 70 X 10^6 (12 injections) sporozoites. This animal remained negative for over 6 months in spite of 5 separate rechallenges in the interim. It will be challenged by parasitized blood to ascertain if the observed protection was stage specific.

Other attempts utilizing similar vaccination schemes to immunize 2 Rhesus monkeys by the subcutaneous route and "by bites" of X-irradiated mosquitoes infected with P. knowlesi proved unsuccessful.

All monkeys immunized with attenuated sporozoites produced CSP antibodies of varying titers but these titers were not directly correlated with protection. Non-resistant animals developed a blood infection without any significant delays in patency.

B. Transfer Factor. Dialyzable transfer factor was prepared from peripheral leukocytes of the Rhesus monkey shown to be resistant to repeated rechallenges of sporozoites. Similar preparations were made from a non-immunized monkey. These preparations were given along with X-irradiated sporozoites I.V. to each of 2 pairs of non-immunized Macaca fascicularis monkeys at 2 week intervals. None of the 4 recipient animals were protected from a subsequent sporozoite challenge.

Continued work in the immediate future on the simian model is not anticipated. More emphasis will be placed on understanding the invasive and immune aspects underlying sporozoite-induced infections in rodent malaria.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00110-04 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Gametogenesis and Gamete-Specific Immunity in Malaria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. W. Gwadz	Research Entomologist	LPD NIAID
	R. Carter	Visiting Research Associate	LPD NIAID
	M. M. Nijhout	Staff Fellow	LPD NIAID
Other:	S. K. Martin	Visiting Fellow	LPD NIAID
	L. H. Miller	Head, Malaria Section	LPD NIAID
	D. H. Chen	Staff Fellow	LPD NIAID
	I. Green	Medical Director (Research)	LI NIAID

COOPERATING UNITS (if any)

M. Aikawa, Case Western Reserve University, Cleveland, Ohio.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
56/12	32/12	24/12

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to investigate the vertebrate host factors which regulate gametogenesis of malaria parasites of the genus Plasmodium and to develop methods for immunizing the vertebrate host against the sexual stages of the parasite which develop in the gut of the mosquito vector.

Studies underway are concerned with: 1) biological and biochemical factors which regulate gametocyte maturation and exflagellation; 2) vertebrate factors which enhance or inhibit exflagellation, fertilization and oocyst development in the mosquito gut; 3) development of an efficient method for immunization of the vertebrate host against the sexual stages of the malarial parasite.

Project Description:

To date, studies have been confined to the avian malaria, Plasmodium gallinaceum, in the chicken, with Aedes aegypti as the mosquito vector.

Gametogenesis

By studying gametocyte carrying P. gallinaceum infected chicken blood in gas tight chambers (Dvorak-Stotler chambers) we have shown that in bicarbonate containing saline solutions, microgamete formation (exflagellation) is controlled solely by changes in the pH of the medium. Below pH 7.6 exflagellation is totally suppressed; maximum levels of exflagellation are achieved at pH 7.8 to 8.2. In the absence of bicarbonate, exflagellation is suppressed at all pH levels.

Current investigations are directed towards clarifying the mechanisms of the bicarbonate dependent pH control of gamete formation, particularly the possible involvement of ion flow and ion exchange between cellular and extracellular compartments. Various cytological studies by light and electron microscopy are in progress to clarify the structural changes involved in gamete formation.

Gamete-specific immunity

Chickens have been immunized with various antigen preparations derived from infected erythrocytes or purified gametes. Once immunized, chickens are fully susceptible to challenge infections, develop asexual parasitemias comparable to infections in non-immune chickens and produce normal numbers of gametocytes. However, immunization has a dramatic effect on the infectivity of gametocytes to mosquitoes. Mosquito-ingested gametocytes from chickens which have received 2 or more inoculations produce few oocysts, whereas gametocytes from once-inoculated or non-immunized control chickens produce heavy mosquito-gut infections. Gametocytes from chickens immunized with 3 weekly injections of 10^7 purified microgametes fail completely to infect mosquitoes.

The reaction which prevents oocyst development is restricted to the mosquito gut and is related to the immobilization of gametes within the mosquito gut by vertebrate host antibodies. In blood from immunized chickens, exflagellation is initiated, but microgamete activity is arrested within seconds. Fertilization of macrogametes is prevented and oocysts do not develop.

Immunization does not affect the functional integrity of circulating gametocytes before ingestion by mosquitoes. Erythrocyte-cloaked gametocytes from immunized chickens, after washing and resuspension in normal serum, are fully infectious to mosquitoes, whereas gametocytes resuspended in autologous immune serum fail to infect.

Various experiments have demonstrated that the gamete-immobilizing activity of immune serum is associated with the IgG fraction and is not complement dependent.

Significance to Biomedical Research

A vaccine capable of interrupting the transmission of malaria by interrupting the sexual cycle of the parasite and preventing mosquito infection could have a profound effect on the epidemiology of one of mankind's major diseases.

Proposed Course

The following investigations will be pursued in the coming year with the eventual goal of immunization against the sexual stages of the human malarias: 1) development of more effective antigens or antigen combinations, more efficient courses of immunization, and evaluation of various adjuvants; 2) determination of the precise mode of action of the anti-gamete response; 3) expansion of studies on gamete-specific immunity to the rodent and simian malaria; 4) continuation of studies on gametogenesis as a prerequisite for antigen mass production.

Publications:

Omar, M.S., Gwadz, R.W., and Miller, L.H.: Incorporation of nucleic acid precursors by Plasmodium cynomolgi in Anopheles balabacensis. Tropenmed. Parasit. 26: 303-306, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00111-04 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1975

TITLE OF PROJECT (80 characters or less)

Genetics of Susceptibility of Anopheline Mosquitoes to Human and Simian Malarias

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.W. Gwadz Research Entomologist LPD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project was suspended early in the report period but will be reactivated at a later date and will be included in project number Z01 AI 00110.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00149-01 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiology of Anopheles Mosquitoes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Frederik Nijhout Staff Fellow LPD NIAID
Mary M. Nijhout Staff Fellow LPD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
36/12	18/12	18/12

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate aspects of the physiology of Anopheles mosquitoes that are of particular interest in their capacity as vectors of malaria. Four lines of investigation that are being pursued at present are: 1) Investigation of the factors that mediate sexual attraction of mosquitoes; 2) Histological studies of the development of the ovary; 3) The endocrine control of ovarian maturation; 4) The physiology of blood feeding of female mosquitoes.

PHS-6040

Project Description:

The objective of this project is to conduct fundamental studies on the physiology of anopheline mosquitoes. Emphasis has currently been placed on reproductive and feeding physiology.

Reproductive physiology and Anopheles males

Males of many species of mosquitoes are sexually attracted to the female flight sound. Sound reception is mediated by the male's antennae. In Anopheles males the long antennal hairs that receive the sound signal are normally closely appressed to the antennal shaft during the daytime and presumably unable to perceive sound. Such males exhibit no mating activity. At night the antennal hairs are erected to stand perpendicular to the antennal shaft, in this configuration the male can detect a female and mating takes place. In Anopheles antennal erection has the character of a circadian rhythm.

Erection of the antennal hairs is under nervous control and the neurotransmitter involved has been tentatively identified as a biogenic amine (probably a catecholamine). The response to exogenously applied catecholamines is inhibited by α-adrenergic blocking agents but not by β-adrenergic blocking agents, suggesting that the receptor in the antennal hair erection effector resembles the vertebrate α-adrenergic receptor.

Cyclic nucleotides (cyclic AMP and cyclic GMP) are able to mimic the action of catecholamines. Thus, it seems likely that induction of antennal hair erection is mediated by an increased level of cyclic nucleotides in the effector cells.

Experiments are now underway to identify (histologically) the effector mechanism, to localize and identify the biogenic amines that cause antennal hair erection, and to identify the pathway by which their release is controlled.

Reproductive physiology of Anopheles females

A histological study of egg development in Anopheles freeborni females has identified the various tissues of the ovaries and followed their changes as the eggs develop after a blood meal. Of significance to anopheline physiology are the morphological changes that occur in the follicular epithelium at the time of chorion (egg shell) formation. In the formation of the lateral floats that characterize Anopheles eggs, the follicle cells form a bilayer and the chorion is molded between them whereas the chorion is secreted directly onto the egg surface in other areas of the oocyte. The trophic nurse cells of the oocyte contain giant or polytene chromosomes, distinguishing Anopheles ovaries from those of all other groups of insects. These chromosomes are easily identified in the half-developed ovary. They are present in the nurse cells of the immature ovary even before the female takes a blood meal. A study to correlate the DNA elaboration in nurse cell nuclei with stages of ovarian development using routine stain and squash preparations combined with autoradiography following tritiated thymidine incorporation is planned.

Anopheles mosquitoes share with other hematophagous insects an intimate relationship between adult blood feeding and egg maturation. In mosquitoes, ovarian development has best been studied in Aedes agypti, a common laboratory animal. Briefly, the distension of the gut with a protein meal acts in some way to cause the release of a neurosecretory factor from the brain. This is a tropic factor for the ovaries and induces them to secrete ecdysone, the insect steroid hormone. Ecdysone stimulates the synthesis of a specialized yolk protein by the cells of the fat body tissue which is released into the hemolymph and transported into the oocytes. Experiments with Anopheles freeborni indicate that the regulation of ovarian development is very similar to that in Aedes. Thus this aspect of the project has been discontinued in order to study anopheline-specific phenomena.

Feeding physiology of female mosquitoes

Adult female mosquitoes are known to take nourishment from two distinct sources: 1) blood from a vertebrate host, the proteins from which are utilized in the production of eggs and 2) sugar solutions from plant nectaries, used primarily for flight energy. These two types of food are taken into distinct regions of the digestive system. Blood is dispatched directly to the midgut (stomach) for immediate digestion whereas sugar solutions are stored in a diverticulum of the esophagus and gradually passed to the stomach for digestion. This sugar storage is presumably adaptive, leaving the stomach empty to receive a blood meal (an unpredictable event) at any time. The purpose of the present study is to examine the mechanism by which a female mosquito recognizes the nature of a meal.

Our results to date show that, in addition to the nature of the meal (sugar or blood), the temperature and the method by which it is offered to the female are of considerable importance in determining the destination of the meal. A warm sugar solution, fed through a membrane goes largely to the gut whereas cold blood from an exposed droplet is dispatched to the diverticulum. Thus the conditions under which a meal is offered are at least as important for proper recognition by the mosquito as the nature of the ingested substance.

Other experiments have demonstrated that additional factors (pH; relative concentrations of sugar and blood components; "hunger"; presence of phagostimulants such as ATP) can modulate the response of the female to the two parameters mentioned above. Experiments are now underway to further characterize the contribution of these various factors to meal recognition and the pathway by which recognition is transferred to the sphincter muscles that control the destination of a meal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00150-01 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Enzyme Variation in Malaria and Babesia Parasites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Carter	Visiting Associate	LPD NIAID
Other:	L. H. Miller	Head, Malaria Section	LPD NIAID
	T. Shiroishi	Zoologist	LPD NIAID

COOPERATING UNITS (if any)

A. Spielman, Department of Tropical Public Health, Harvard Medical School.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
22/12	4/12	18/12

SUMMARY OF WORK (200 words or less - underline keywords)

Electrophoretic variation of enzymes of malaria and babesia parasites is being studied by starch gel electrophoresis. Material for the study of the human malaria parasites P. vivax and P. falciparum has been collected and is stored ready for examination. An expedition to the island of Nantucket has been planned for the collection of isolates of babesia from rodents. Isolates of human babesia are already stored ready for examination. Four enzymes have been found to be suitable for the study of enzyme variation in malaria parasites and two are suitable for study in babesia.

Project Description:

The distribution of electrophoretic variation of enzymes in isolates of human malaria parasites Plasmodium vivax and Plasmodium falciparum from various parts of the world and of Babesias of both rodent and human origin from the island of Nantucket is being investigated. Although the parasite material for this study is still being collected most of the isolates of human malaria have been grown up in Aotus monkeys and are stored as freeze dried material ready for testing. Likewise most of the available isolates of Babesia from humans have been prepared and await testing. An expedition to the island of Nantucket to collect isolates of Babesia from the local rodent populations has been organized.

Preliminary investigations have shown that four enzymes, glucose phosphate isomerase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase and glutamate dehydrogenase may be readily studied in preparations of blood stage malaria parasites on starch gel electrophoresis. So far only glucose phosphate isomerase and glutamate dehydrogenase have been identified in Babesia preparations by this method.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00113-04 LPD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Dengue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. Dr. Leon Rosen, Head, Pacific Research Section, LPD, NIAID

Other: Dr. T. T. Kuberski, Staff member, Pacific Research Section LPD, NIAID
Dr. Dwayne Reed, Epidemiologist, South Pacific Commission, Noumea, New Caledonia
Dr. Jona Mataika, Director, Wellcome Virus Laboratory, Fiji
Dr. Henri Kaeuffer, Staff member, Institut de Recherches Medicales "Louis Malarde", Papeete, Tahiti

COOPERATING UNITS (if any)

Departments of Health, American Samoa, Western Samoa, Niue, Tonga, Fiji, Guam, New Hebrides, Indonesia, Philippines, and U.S. Center for Disease Control.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Pacific Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Honolulu, Hawaii

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.4	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

Epidemiologic studies of recent dengue outbreaks on Pacific islands have shown that severe and fatal disease can result from a first infection with a dengue virus. These observations are not in accord with the widely held belief that such disease results only from a second or third dengue infection and is the result of some sort of "immunologic enhancement". The epidemiologic studies also have shown that both dengue types 1 and 2 can be propagated in nature by Aedes polynesiensis in the absence of the classical vector Aedes aegypti. In laboratory studies it was shown that Toxorhynchites amboinensis, a very large and hardy species of mosquito which does not feed on blood, was an excellent laboratory host for the detection and propagation of dengue and several other arthropod-borne viruses.

PHS-6040

Project Description:

Objectives

1. To study the epidemiology of dengue and dengue hemorrhagic fever.

2. To investigate the pathogenesis of severe and fatal dengue infection.

3. To improve laboratory methods for the detection of dengue viruses and their antibodies.

Methods Employed

Investigations on the epidemiology of dengue, and to a large extent those on its pathogenesis, are dependent on the opportunities presented by naturally occurring outbreaks of the disease. During the past year dengue type 1 has continued to spread to various island groups in the South Pacific. This has afforded an opportunity to evaluate the role of mosquito species other than Aedes aegypti in the transmission of the disease and to obtain data on the respective roles of primary and secondary dengue infections in the pathogenesis of severe and fatal disease. In contrast to situations on Pacific islands, dengue in Southeast Asia is largely endemic in nature and occurs among very large populations. This affords an opportunity to collect strains of differing serotypes for virulence studies and to study the rarer forms of severe and fatal dengue disease.

Major Findings

A major outbreak of type 1 dengue infection with a relatively high incidence of hemorrhagic manifestations occurred in Fiji in a population which had experienced a less extensive type 2 dengue outbreak several years previously. This afforded an opportunity to compare the nature of disease in persons experiencing their first dengue infection as contrasted with those experiencing their second. Severe bleeding appeared to have occurred as frequently in primary infections as in secondary infections. Hypocomplementemia was observed in both primary and secondary dengue and appeared to be related to severity of disease rather than to primary or secondary infection.

Outbreaks of dengue type 1 infection also were observed in Tonga, Tahiti, American and Western Samoa, and the Cook Islands. Hemorrhagic disease was common and a number of deaths attributed to dengue occurred in Tonga, but little or no hemorrhagic disease and no deaths were reported from the other island territories. There was no obvious explanation for this difference in pathogenicity. Dengue type 1 virus was isolated from a fatal case of dengue shock syndrome on Tonga and antibody studies

indicated that the infection was primary. Thus, it has now been demonstrated that dengue type 1, as well as types 2 and 3 can lead to fatal disease in persons previously unexposed to dengue viruses. In the recent prior spread of dengue type 2 throughout the South Pacific Tonga experienced the least extensive outbreak and the one characterized by very mild disease. The differences in the two recent dengue outbreaks which occurred in that territory were quite striking.

Dengue did not become established on Guam despite the sudden introduction of thousands of Vietnamese refugees. A number of severe dengue infections and one fatality were recognized among the latter individuals (all acquired in Vietnam). Aedes aegypti does not occur on the island but Aedes albopictus and another Stegomyia species, Aedes guamensis, are common. Because of the extensive anti-mosquito measures undertaken at the time it could not be determined whether or not these mosquitoes were suitable vectors. Dengue virus was isolated from the liver of the fatal case on Guam by means of the mosquito isolation technique. Since only one liver isolate had previously been obtained from fatal dengue infections despite hundreds of attempts, and the validity of even that isolate has been challenged, it appears that the mosquito isolation technique should be used for further studies of tissue from fatal infections.

Serologic studies carried out on Rarotonga, Cook Islands indicated that a few dengue type 2 infections had occurred there in the recent past in the absence of Aedes aegypti. A dengue type 1 outbreak now occurring on the island confirms the ability of the only Stegomyia species on the island, Aedes polynesiensis, to transmit both dengue type 1 and type 2.

Collection of dengue virus strains for future pathogenesis studies was continued with additional strains obtained from patients from Vietnam, Indonesia, Philippine Islands, Fiji, Niue, Tahiti, Tonga, American Samoa and the New Hebrides.

The isolation of dengue viruses has been greatly facilitated by using parenterally inoculated Aedes albopictus or Aedes aegypti mosquitoes as laboratory hosts. While this procedure has an important advantage in sensitivity over the use of cell cultures or mice for similar purposes, it is not without disadvantages. One of the disadvantages is the relatively small quantity of inoculum which can be injected into a single insect without high mortality. Another is the toxicity of some undiluted human sera for these species of mosquitoes, especially for males. Also, the establishment of laboratory colonies of these two Aedes, or related species, would not be desirable in certain tropical areas where they were not found naturally.

Virologic studies

The collection of dengue strains (including original human sera) from various geographic localities and from patients with various types of dengue disease will be continued for eventual study of biologic properties (e.g. *in vitro* markers of virulence, basic biologic differences among the four serotypes, etc.)

Work also will be continued on several projects designed to improve currently available virologic techniques for dengue. Among these will be a study of various species of *Toxorhynchites* mosquitoes to select the most appropriate for laboratory use with respect to sensitivity to dengue virus infection, ultimate quantity of dengue virus produced, and ease of laboratory colonization. It is anticipated that these very large, non-bloodsucking mosquitoes ultimately will become the standard laboratory animal for the isolation of dengue (and probably many other arthropod-borne) viruses.

Significance to Biomedical Research and the Program of the Institute

Dengue viruses continue to increase in importance as significant causes of morbidity and mortality in Southeast Asia and on Pacific islands - probably because of rapidly increasing urbanization and travel in those areas. Aside from their world-wide civilian and military importance, major outbreaks of infection with these agents have occurred during the past several years in a number of the Pacific island groups which are the responsibility of the United States (American Samoa, Marshall Islands, Caroline Islands, and Guam). Moreover, other U.S. territory is susceptible to the establishment of dengue infection and has experienced such infection in the past (Hawaii, Puerto Rico, much of the Southern continental United States). Obviously, further understanding of the epidemiology of dengue viruses and of the pathogenesis of the diseases they produce would be of value.

In an attempt to overcome these difficulties we explored the use of mosquitoes of the genus Toxorhynchites for the isolation and propagation of dengue viruses. Toxorhynchites is a genus of unusually large and hardy mosquitoes in which the adults do not feed on blood.

It was found that both sexes of Toxorhynchites amboinensis were as susceptible to infection with each of the four prototype strains of dengue virus as was A. albopictus. As judged by measurement of complement-fixing antigen, infected T. amboinensis apparently contain at least as much virus as infected A. albopictus. It also was found that Japanese encephalitis and yellow fever (17D) viruses replicate in Toxorhynchites, suggesting that this genus also may prove useful for the study of other arthropod-borne viruses.

Proposed Course of the Project

Epidemiologic studies

Investigations will be undertaken in Rangoon and possibly other cities in Burma with two principal objectives in mind. One will be an attempt to evaluate the risk of a primary dengue infection as compared with a secondary dengue infection with respect to the development of the shock syndrome or severe bleeding. The other will be an attempt to evaluate the comparative risk of infection with each of the four dengue serotypes with respect to the clinical phenomena mentioned.

Clinical studies

A study will be undertaken of the presence or absence of dengue virus in various organs of patients dying of dengue employing sensitve mosquito isolation techniques. Material will be obtained primarily in Rangoon, where a large number of fatal cases occur and autopsies are performed on most patients. However, material also will be collected in Jakarta and possibly other cities in Indonesia by means of post-mortem liver and brain needle biopsies.

Studies will be continued on the relationship between the magnitude of dengue viremia and magnitude and nature of dengue antibody response to clinical severity of dengue. This will be an attempt to determine if severe dengue is associated with higher than average viremia or higher than average or unusual types of antibody response.

Yii, C-Y., Chen, C-Y., Chen, E-R., Hsieh, H-C., Shih, C-C., Cross, J.H. and Rosen, L.: Epidemiologic studies of eosinophilic meningitis in southern Taiwan. Am. J. Trop. Med. Hyg. 24: 447-454, 1975.

Kaeuffer, H., Rosen, L. and Laigret, J.: Surveillance séro-épidémiologique de la dengue à Tahiti. Méd. Mal. Infect. 5:549-550, 1975.

Gubler, D.J. and Rosen, L.: A simple technique for demonstrating transmission of dengue virus by mosquitoes without the use of vertebrate hosts. Am. J. Trop. Med. Hyg. 25:146-150, 1976.

Gubler, D.J. and Rosen, L.: Variation among geographic strains of Aedes albopictus in susceptibility to infection with dengue viruses. Am. J. Trop. Med. Hyg. 25:318-325, 1976.

Kuberski, T.T. and Rosen, L.: Erythema Infectiosum. In Top, F.H., Sr. and Wehrle, P.F. (eds.): Communicable and Infectious Diseases, 8th edition (in press)

Rosen, L., Rozeboom, L.E., Reeves, W.C., Saugrain, J. and Gubler, D.J.: A field trial of competitive displacement of Aedes polynesiensis by Aedes albopictus on a Pacific atoll. Am. J. Trop. Med. Hyg. (in press)

Gubler, D.J. and Rosen, L.: Quantitative aspects of replication of dengue viruses in Aedes albopictus after oral and parenteral infection. J. Med. Entomol. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00114-06 LPD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the arboviruses of the Phlebotomus fever serogroup

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert B. Tesh, Staff Member, Pacific Research Section, LPD, NIAID

Other: Leon Rosen, Head, Pacific Research Section, LPD, NIAID
S. Saidi, School of Public Health and Institute of Public Health Research, University of Teheran, Iran
G. Papaevangelou, Department of Hygiene & Epidemiology University of Athens Medical School, Greece

COOPERATING UNITS (if any) Institut Pasteur, Paris, France; Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Connecticut; US Naval Medical Research Unit #2, Taipei, Taiwan; US Naval Medical Research Unit #3, Cairo Egypt; Dept. of Tropical Medicine & Medical Microbiology, University of Hawaii School of Medicine

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Pacific Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Honolulu, Hawaii

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

Field studies on the epidemiology of sandfly fever were carried out in 5 villages in central Iran. From Phlebotomus papatasi collected in the villages, 60 isolates of Sicilian and Karimabad viruses were obtained. Many of the human inhabitants of the study area had neutralizing antibodies against these two viruses. Host identification studies on 575 blood-engorged P. papatasi collected in the villages indicated that they had fed on chickens, pigeons, humans, bovines, equines, sheep and goats.

Several thousand human sera from Africa, the Mediterranean region, Central and Southeast Asia were screened by plaque reduction neutralization method against 8 Phlebotomus fever virus serotypes occurring in the Old World. Results of these studies indicate that Naples and Sicilian virus infection is common among residents of North Africa, the Mediterranean and Central Asia. Karimabad virus neutralizing antibodies were found only in Iran. Antibodies against the other 5 Phlebotomus fever serotypes were uncommon, suggesting that the latter agents infrequently infect man and are of little public health importance.

PHS-6040
(12-75)

Project Description:

Objectives

1. To study the epidemiology of sandfly fever among human populations living in endemic areas of the disease.

Methods Employed

Primary isolation of viruses from insect specimens was done in tube cultures of Vero cells. Identification of virus isolates was done by complement fixation or neutralization test. Serologic studies of human and animal sera were performed by standard complement fixation or plaque reduction neutralization tests. Identification of insect blood meals was done by precipitin test.

Major Findings

A. Effect of insecticide spraying for malaria control on the incidence of sandfly fever in Athens, Greece

Sera from 637 Athens residents of various age groups were examined by plaque reduction neutralization test for antibodies against Naples and Sicilian Phlebotomus fever viruses. A marked change in the prevalence of antibodies to both agents was observed in persons born after 1946, when residual insecticide spraying for malaria control was initiated in Greece. The prevalence of Naples and Sicilian neutralizing antibodies among residents $\geq$30 years of age was 36 and 13%, respectively. In contrast, only 4% of persons $\leq$29 years of age had Naples antibodies and all were negative to Sicilian. These serologic data confirm previous clinical observations that sandfly fever became uncommon in Athens after initiation of the insecticide spraying program. Presumedly the spraying program was effective in reducing the Phlebotomus population to a level where virus transmission was minimal. Results of this study also demonstrated that neutralizing antibodies to the Naples and Sicilian virus types persist for at least 30 years, suggesting that immunity to sandfly fever virus infection is long-lasting.

B. Field studies on the epidemiology of sandfly fever in Iran

During the summer of 1975, field studies on the epidemiology of sandfly fever were conducted in 5 villages in Isfahan Province, Iran. A total of 12,485 Phlebotomus papatasi (both males and females) were collected from houses in the villages and were processed for virus isolation in tube cultures of Vero cells. From these insect specimens, 62 virus isolates were obtained. Two isolates were made from pools of male sandflies; the remainder were from females. The overall virus isolation rate obtained from female P. papatasi was 1 per 177 insects processed, demonstrating the high vector potential of this species. Identification of the sandfly isolates indicated that 49 were

Sicilian virus, 11 were Karimabad (both members of the Phlebotomus fever arbovirus group), and 2 were an apparent new member of the vesicular stomatitis virus serogroup, designated as Isfahan virus.

Blood specimens were obtained from humans, domestic animals and gerbils in the study area and were examined by plaque reduction neutralization test for antibodies against 4 Phlebotomus fever virus serotypes (Naples, Sicilian, Karimabad and Salehabad), known to occur in Iran. Many of the human residents of the study area had neutralizing antibodies to Sicilian and Karimabad viruses. A smaller number, mostly older persons, had evidence of Naples virus infection. Of the animal sera tested, only gerbils (Rhombomys optimus) showed evidence of infection. Both Sicilian and Karimabad neutralizing antibodies were found in gerbil sera, suggesting that these rodents might serve as amplifying hosts of the viruses. Studies on the experimental infection of gerbils with these two viruses are now in progress to determine the level and duration of viremia.

Blood meals from 575 P. papatasi collected in the villages were examined by microplate precipitin test. The majority of the sandflies (58%) had fed on birds, mainly chickens and pigeons. The remainder had fed on humans, equines, bovines, sheep and goats. The results suggest that P. papatasi is an opportunistic feeder and is not strongly anthropophilic.

C. Characterization and studies on the ecology of Isfahan virus

During field studies on the epidemiology of sandfly fever in Iran, 2 isolates of Isfahan virus, an apparent new member of the vesicular stomatitis serogroup, were obtained from P. papatasi. The new agent is chloroform sensitive and by electron microscopic examination shows typical rhabdovirus morphology. Its cytopathic effect, growth characteristics, plaque morphology and pathogenicity for mice are typical of the VSV group. By complement fixation and neutralization tests, Isfahan virus is distinct from the 6 known VSV serotypes, although it reacts with a VSV grouping antiserum. Serologic studies on human sera from various regions of Iran indicate that many persons have high titers of neutralizing antibodies to the virus. In the village in which the virus was isolated, 100% of the inhabitants over 5 years of age have been infected. Although the human disease potential of Isfahan virus is unknown, apparently infection is common among persons living in rural areas of Iran.

D. Geographic distribution and prevalence of human infection with Phlebotomus fever group viruses in the Old World

Several thousand human sera from various regions of Arica, the Mediterranean region, Central and Southeast Asia were screened by plaque reduction neutralization method against 8 Phlebotomus fever virus serotypes occurring in the Old World. Results of these studies indicate that only the Naples, Sicilian and Karimabad serotypes are of significant public health

importance. Our studies show that the Naples and Sicilian neutralizing antibodies are common among inhabitants of North Africa, the Mediterranean region and Central Asia but are absent from residents of Central Africa, Southeast Asia and China. The distribution of these two virus serotypes appears to closely parallel the geographic distribution of P. papatasi, their presumed vector. In contrast, human infection with Karimabad virus seems to be restricted to central and northeast Iran. The purpose of this study is to eventually map the distribution and prevalence of the known Phlebotomus fever serotypes in the Old World.

E. The prevalence of West Nile virus human infection in Iran

A total of 698 blood and serum specimens from residents of 13 Iranian communities were examined by plaque reduction neutralization test for antibodies to West Nile virus. Results of this study indicate that West Nile virus is widely distributed in Iran. The highest prevalence of infection (60-95%) was found among residents of Khuzestan Province (Southwest Iran). This is the major oil-producing region of the country. It is also an area of intensive agriculture with water supplied by numerous irrigation canals. Presumedly the canals and their runoff provide suitable breeding places for large numbers of Culex mosquitoes, the probable vector of West Nile virus. This situation is somewhat analagous to the mosquito problem created by intensive agriculture and irrigation in the San Joaquin and Imperial Valleys of California.

Significance to Biomedical Research and the Program of the Institute

Although sandfly fever is presumed to be endemic in many areas of the Middle East and Central Asia, little is known about the epidemiology of the disease among indigenous populations. Previous studies of sandfly fever have been conducted mainly on foreign military personnel stationed in the region, consequently these investigations have been limited to the clinical manifestations of the disease, attack rates and mode of transmission. A number of basic facts about the epidemiology of sandfly fever are still unanswered, such as the true prevalence of infection among local populations, the clinical spectrum of the disease, the prevalence of different virus serotypes, the sandfly species actually involved in virus transmission, the maintenance mechanism of the virus from season to season, and the possibility of an animal reservoir. Answers to these questions have been difficult to obtain mainly because of problems in isolating Phlebotomus fever viruses and the lack of a specific serologic test to detect infection.

Two recent advances in laboratory techniques for working with the Phlebotomus fever group, the use of tissue culture for primary virus isolation and the development of a specific plaque neutralization test for detecting sandfly fever antibodies, have provided the necessary tools for answering some of the forementioned questions. In 1975 at the invitation

of the Iranian government, field studies were carried out in several rural villages in central Iran where sandfly fever is endemic. These studies were a collaborative effort between the School of Public Health, University of Teheran and the Pacific Research Section, LPD, NIAID. The studies were designed to provide new information on the epidemiology of sandfly fever.

Because of its clinical similarity to foot-and-mouth disease, vesicular stomatitis has long been of interest to veterinary and animal health personnel. Although little is known about the ecology of vesicular stomatitis virus (VSV), studies in the Central America and Panama indicate that human infection with VSV is also common. Experimental infections of laboratory personnel working with the virus suggest that it produces an influenza-like illness in man. Six distinct VSV serotypes are currently known. Five occur in the Americas (two in the United States) and the sixth has been isolated in India and Nigeria. Two of the known VSV serotypes have been associated with phlebotomine sandflies, suggesting that these insects may serve as vectors of the viruses. The recovery of a seventh VSV serotype (Isfahan virus) from Phlebotomus papatasi in Iran is therefore of interest. The high prevalence of Isfahan virus neutralizing antibodies observed among rural inhabitants of Iran suggest that this virus, like the Indiana, New Jersey and Chandipura VSV serotypes, may be of considerable public health importance.

Proposed Course of the Project

Additional studies on the epidemiology of sandfly fever are planned. One question, yet to be answered, is the possibility of an animal reservoir. The finding that gerbils are naturally infected with Phlebotomus fever viruses, suggests that these rodents may serve as amplifying hosts of the viruses. To test this possibility, further collaborative studies with our Iranian collaborators are planned. During the Spring they will collect young gerbils and infect them with several Phlebotomus fever viruses to determine the level and duration of viremia. Subsequent laboratory work will be done in Honolulu. Further serologic studies are also planned in order to map the distribution and prevalence of Phlebotomus fever virus infection in the Old World.

Publications

Tesh, R.B.: Multiplication of Phlebotomus fever group arboviruses in mosquitoes after intrathoracic inoculation. J. Med. Entomol. 12:1-4, 1975.

Tesh, R.B., Gajdusek, D.C., Garruto, R.M., Cross, J.H., and Rosen, L.: The distribution and prevalence of group A arbovirus neutralizing antibodies among human populations in Southeast Asia and the Pacific islands. Am. J. Trop. Med. Hyg. 24:664-675, 1975.

Tesh, R.B., and Gubler, D.J.: Laboratory studies of transovarial transmission of La Crosse and other arboviruses by Aedes albopictus and Culex fatigans. Am. J. Trop. Med. Hyg. 24:876-880, 1975.

Tesh, R.B., and Chaniotis, B.N.: Transovarial transmission of viruses by phlebotomine sandflies. Ann. N. Y. Acad. Sci. 266:125-134, 1975.

Tesh, R. B., and Rosen, L.: Failure of rubella virus to replicate in mosquitoes. Intervirology 5:216-219, 1975.

Tesh, R.B., Gubler, D.J., and Rosen, L.: Variation among geographic strains of Aedes albopictus in susceptibility to infection with chikungunya virus. Am. J. Trop. Med. Hyg. 25:326-335, 1976.

Bartelloni, P.J., and Tesh, R.B.: Clinical and serologic responses of volunteers infected with Phlebotomus fever virus (Sicilian type). Am. J. Trop. Med. Hyg. 25:456-462, 1976.

Saidi, S., Tesh, R., Javadian, E., and Nadim, A.: The prevalence of human infection with West Nile virus in Iran. Iranian J. Publ. Hlth. 1976 (in press).

Tesh, R., Saidi, S., Javadian, E., Nadim, A., and Sahabi, Z.: The distribution and prevalence of human infection with Phlebotomus fever group viruses in Iran. Iranian J. Publ. Hlth. 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI 00115-02 LPD

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Natural History of Influenza Virus in Swine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. D. Wallace, Staff Member, Pacific Research Section, LPD, NIAID

COOPERATING UNITS (if any)

Animal Science Department, College of Tropical Agriculture, University of Hawaii and Division of Animal Industry, Hawaii State Department of Agriculture, Honolulu, Hawaii

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Pacific Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Honolulu, Hawaii

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The NATURAL HISTORY of SWINE INFLUENZA is being studied retrospectively and prospectively by standard serologic and virologic techniques in swine herds in Hawaii. The objectives are to determine how swine influenza virus is maintained in nature between epidemics, what factors are critical for the occurrence of epidemics in swine, and to make observations on the interchange of influenza virus between swine and man. Antibody surveys indicate that swine influenza virus (HswlN1) is endemic in swine on Oahu, Hawaii. Some herds have been found with antibody to current human strains of influenza virus (H3N2), but transmission of these strains in swine appear to be limited. To date, the data obtained are compatible with the hypothesis that swine influenza virus is maintained in certain herds, in the absence of LUNGWORMS, through chronic or LATENT INFECTIONS in individual animals. Conditions critical for the occurrence of influenza epidemics in swine in Hawaii are still unclear.

PHS-6040
(12-75)

PROJECT DESCRIPTION:

OBJECTIVES

TO DETERMINE: 1) HOW SWINE INFLUENZA VIRUS IS MAINTAINED IN NATURE BETWEEN EPIDEMICS IN SWINE. 2) FACTORS CRITICAL TO EPIDEMIC SPREAD OF INFLUENZA VIRUS IN SWINE, 3) FREQUENCY AND EPIDEMIOLOGIC IMPORTANCE OF INTERCHANGE OF INFLUENZA VIRUSES BETWEEN SWINE AND MAN.

METHODS EMPLOYED

OBSERVATIONS ON THE EPIDEMIOLOGY OF INFLUENZA IN SWINE INDICATE THAT SWINE INFLUENZA VIRUS IS MAINTAINED IN SWINE HERDS BY METHODS OTHER THAN PERIODIC REINTRODUCTION FROM THE OUTSIDE. DR. RICHARD SHOPE DEVELOPED THE CLASSIC HYPOTHESIS, SUPPORTED BY A LARGE SERIES OF EXPERIMENTS, THAT SWINE LUNGWORM LARVAE WHILE LIVING IN THEIR EARTHWORM HOST SERVE AS AN EXTRA-MAMMALIAN RESERVOIR OF VIRUS. OTHER DATA, HOWEVER, FROM EXPERIMENTS AND EPIDEMIOLOGIC OBSERVATIONS CONDUCTED IN CZECHOSLOVAKIA, SUPPORTED BY INDEPENDENT EPIDEMIOLOGIC OBSERVATIONS IN WISCONSIN, USA, SUGGEST THAT SOME SWINE MAY BECOME CHRONICALLY INFECTED IN THE ABSENCE OF LUNGWORMS AND PERIODICALLY SHED VIRUS OVER A LONG PERIOD OF TIME. IT WAS SHOWN EXPERIMENTALLY THAT PIGS BORN TO SOWS INFECTED DURING PREGNANCY, OR BORN TO CHRONICALLY INFECTED SOWS IN WHICH INFECTION IS ACTIVATED DURING PREGNANCY, DEVELOP IMMUNOLOGIC TOLERANCE AND HARBOR DEMONSTRABLE VIRUS THEMSELVES FOR LONG PERIODS.

THESE HYPOTHESES (LUNGWORM TRANSMISSION, AND CHRONIC SHEDDING OF VIRUS), WERE DEVELOPED PRIMARILY FROM EXPERIMENTAL DATA AND ARE NOW BEING TESTED IN HAWAII AND ON OTHER PACIFIC ISLANDS BY CAREFUL LONG-TERM PROSPECTIVE SERO-EPIDEMIOLOGIC AND VIROLOGIC STUDY OF NATURALLY INFECTED SWINE HERDS. THIS CAN BE ACCOMPLISHED TO ADVANTAGE ON PACIFIC ISLANDS BECAUSE OF RELATIVE ISOLATION, AND THE AVAILABILITY OF SWINE HERDS INFECTED WITH INFLUENZA IN THE ABSENCE OF LUNGWORMS.

MAJOR FINDINGS

RESULTS OF HEMAGGLUTINATION-INHIBITION (HI) TESTS ON SERA COLLECTED FROM 2-- YEAR OLD SWINE AT SLAUGHTER IN 1971 (REPORTED BY OTHERS), 1974 AND 1975 INDICATED THAT A/SWINE (HSW1N1) INFLUENZA VIRUS WAS ENDEMIC IN SWINE ON THE ISLAND OF OAHU. IN ADDITION, HI ANTIBODY TO A/HONG KONG/8/68 (H3N2) VIRUS WAS PRESENT IN SWINE SERA COLLECTED IN 1974 AND 1975 FROM A NUMBER OF HERDS ON OAHU. HI TESTS ON SERA COLLECTED IN 1976 FROM 204 1-2 YEAR OLD BREEDING SOWS IN 27 HERDS ON OAHU NOT TESTED PREVIOUSLY REVEALED THAT 33% AND 15% OF THE HERDS HAD ANIMALS WITH ANTIBODY TO A/SWINE AND A/PORT CHALMERS/11/73 (H3N2) VIRUSES, RESPECTIVELY. (A/PORT CHALMERS [A/PC] A VARIANT OF A/HONG KONG HAD BEEN EPIDEMIC IN THE HUMAN POPULATION IN HAWAII.) OF SERA COLLECTED AT SLAUGHTER FROM 247 MARKET HOGS 4-6 MONTHS OF AGE FROM 27 DIFFERENT FARMS

ON OAHU IN MARCH AND APRIL, 1976, SERA FROM 2 ANIMALS FROM ONE FARM WERE FOUND TO HAVE ANTIBODY TO A/SWINE VIRUS, INDICATING THAT A/SWINE VIRUS HAS REMAINED ENDEMIC ON OAHU. ANTIBODY TO A/SWINE VIRUS WAS NOT FOUND IN SERA FROM 270 2-4 YEAR OLD BREEDING SWINE IN 46 HERDS ON 4 OTHER HAWAIIAN ISLANDS. HOWEVER, 8% OF THESE ANIMALS, FROM 6 HERDS, HAD ANTIBODY TO A/PC VIRUS.

DURING THE PAST 18 MONTHS 6 HERDS ON OAHU HAVE BEEN UNDER CLOSE SURVEILLANCE FOR INFLUENZA VIRUS ACTIVITY. THREE HERDS WERE CONSIDERED "CLOSED" SINCE SWINE WERE SELDOM INTRODUCED FROM THE OUTSIDE, AND THEY WERE RELATIVELY ISOLATED FROM OTHER HERDS GEOGRAPHICALLY. AT THE BEGINNING OF THE STUDY MOST OF THE ADULT BREEDING SOWS IN THESE HERDS HAD ANTIBODY TO A/SWINE VIRUS AND ABOUT 50% IN ONE HERD ALSO HAD ANTIBODY TO A/PC VIRUS. HOWEVER SOWS ARE REPLACED AT 3-4 YEARS OF AGE BY YOUNG FEMALES (GILTS) ABOUT 6 MONTHS OF AGE. REPLACEMENT OCCURS CONTINUOUSLY.

BLOOD SAMPLES HAVE BEEN COLLECTED AND TESTED FROM MOST OF THE GILTS AND SOME OF THE SOWS ON EACH OF THE 6 FARMS AT ABOUT 4 TO 6 WEEK INTERVALS. HI TESTS ON SERIAL BLEEDINGS COLLECTED FROM THESE ANIMALS THROUGH ADULT LIFE PROVIDE GOOD SURVEILLANCE OF VIRUS ACTIVITY. THERE HAS BEEN NO SEROLOGIC EVIDENCE OF WIDESPREAD VIRUS ACTIVITY IN ANY OF THE 6 HERDS SINCE THE STUDY BEGAN. IN ONE HERD AT THE BEGINNING OF THE STUDY, 97% OF ALL OF THE ADULT SOWS (ABOUT 250) HAD HI ANTIBODY (TITER $\geq$1:40) TO A/SWINE AND 56% TO A/PC VIRUSES. IN 50% OF 29 POSITIVE SOWS IN THIS HERD REBLED AT 9 TO 11 MONTHS AFTER THE FIRST BLEEDING THERE WAS NO APPRECIABLE DECLINE IN ANTIBODY TITERS TO A/SWINE VIRUS (MANY TITERS REMAINED AT 1:160 TO 1:320); WHEREAS TITERS TO A/PC VIRUS HAD REMAINED STABLE IN ONLY 35%. THE APPEARANCE OF ANTIBODY TO A/SWINE VIRUS WAS DETECTED IN ONE SOW, AND AN 8-FOLD OR GREATER INCREASE IN A/SWINE ANTIBODY TITERS WERE DETECTED IN 3 OTHER SOWS. BY CALCULATING THE PRESENCE OF HI ANTIBODY (TITER $\geq$1:40) BY BIRTH DATE, IT APPEARED THAT AN EPIDEMIC IN THE HERD HAD OCCURRED PRIOR TO SEPTEMBER 1974.

FECES FROM ALL OF THE STUDY HERDS HAVE BEEN EXAMINED FOR LUNGWORM OVA AND NONE HAVE BEEN FOUND. EXAMINATION OF LUNGS AT THE SLAUGHTER HOUSE FOR SEVERAL MONTHS INDICATES THAT VERY FEW SWINE HERDS ON OAHU ARE INFECTED WITH LUNGWORMS.

ATTEMPTS HAVE BEEN MADE TO ISOLATE VIRUS FROM NASAL SECRETIONS OF 42 CLINICALLY NORMAL FEMALE BREEDING SWINE IN 2 OF THE 6 HERDS UNDER SURVEILLANCE. ALL SPECIMENS WERE COLLECTED WITHIN A FEW DAYS OF PARTURITION, TESTING THE HYPOTHESIS THAT THE STRESS OF PARTURITION AND LACTATION MIGHT INDUCE A LATENT INFECTION TO BECOME ACTIVE. NO VIRUSES HAVE BEEN ISOLATED TO DATE, BUT THIS WORK HAS NOT BEEN COMPLETED. CULTURES OF LUNG AND TRACHEA WERE ESTABLISHED FROM 22 NEWBORN SICK PIGS IN THE SAME 2 HERDS MENTIONED ABOVE. THESE PIGS, BORN TO SEROLOGICALLY POSITIVE SOWS, HAD SIGNS AT BIRTH COMPATIBLE WITH CONGENITAL INFLUENZA. VIRUS WAS NOT ISOLATED FROM LUNG TISSUE COLLECTED AT AUTOPSY OR FROM MEDIUM OF ORGAN CULTURES MAINTAINED FOR SEVERAL WEEKS.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

NEW INFORMATION ON THE NATURAL HISTORY OF SWINE INFLUENZA WILL BE OF CONSIDERABLE BIOLOGIC INTEREST AND MAY PROVIDE INSIGHT INTO THE NATURAL HISTORY OF HUMAN INFLUENZA.

PROPOSED COURSE OF THE PROJECT

SEROLOGIC SURVEILLANCE WILL BE CONTINUED BOTH IN THE SELECT STUDY HERDS AND ON SAMPLES OBTAINED FROM SLAUGHTERED ANIMALS FROM OTHER HERDS ON OAHU. EVENTUALLY, EPIDEMICS SHOULD BE ENCOUNTERED PROVIDING OPPORTUNITIES TO ISOLATE VIRUS AND OBSERVE CONDITIONS THAT MIGHT BE CRITICAL TO EPIDEMIC SPREAD OF INFLUENZA VIRUS IN SWINE HERDS. VIRUS STRAINS ISOLATED WILL BE STUDIED ANTIGENICALLY AND USED IN EXPERIMENTS DESIGNED TO EVALUATE VERTICAL TRANSMISSION AND CHRONICITY OF INFECTION.

IN VIEW OF RECENTLY RECOGNIZED HUMAN INFECTIONS WITH AN INFLUENZA VIRUS SIMILAR TO A/SWINE IN NEW JERSEY, PLANS ARE IN PROGRESS TO DETERMINE THE PREVALENCE OF ANTIBODY TO A/SWINE VIRUS IN SWINE FARMERS AND THEIR FAMILIES ON OAHU.

ALSO, THROUGH THE COOPERATION OF LOCAL HEALTH AUTHORITIES, BLOOD SPECIMENS FROM RESIDENT SWINE ON 2 SOUTH PACIFIC ISLANDS ARE BEING COLLECTED. IF THERE IS SEROLOGIC EVIDENCE OF A/SWINE VIRUS ACTIVITY IN THESE ANIMALS, WHICH ARE FREQUENTLY IN CLOSE CONTACT WITH PEOPLE, SPECIMENS FROM HUMAN RESIDENTS OF THE AREAS WILL BE OBTAINED AND TESTED.

PUBLICATIONS: NONE

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00116-02 LPD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Investigations on non-gonococcal urethritis (NGU) and Chlamydia trachomatis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T. T. Kuberski, Staff Member, Pacific Research Section, LPD, NIAID
OTHER: R. Shaw, Epidemiologist, United States Navy, U.S. Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor Naval Shipyard, Honolulu, Hawaii

COOPERATING UNITS (if any)
Hawaii State Venereal Disease Clinic, Honolulu, Hawaii; U.S. Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor Naval Shipyard, Honolulu, Hawaii

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Pacific Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Honolulu, Hawaii

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

Non-gonococcal urethritis (NGU) was found to account for about seventy-five percent of the urethritis seen in males in a civilian venereal disease (VD) clinic population. Chlamydia trachomatis was isolated from the urethra of forty-two percent of men with NGU in this population. The prevalence of antibody to Chlamydia was found to be significantly higher in the VD clinic patients than in a control group. The prevalence of C. trachomatis and other potential pathogens also was studied in a Navy NGU population and a group of comparable controls. This study has recently been completed and the data are currently being analyzed.

PHS-6040
(12-75)

Project Description:

Objectives

The objectives in this study are two-fold:

1. To characterize the epidemiology, clinical and laboratory aspects of non-gonococcal urethritis (NGU), particularly with respect to C. trachomatis.

2. To contribute to the understanding of the epidemiology of C. trachomatis in those human populations where relatively little good information is currently available.

Methods Employed

A pilot study was undertaken at a local civilian venereal disease (VD) clinic to examine male patients with non-gonococcal urethritis. Urethral swabs from 26 NGU patients were cultured for C. trachomatis. Antibody prevalence to C. trachomatis was also examined in 100 random sera from VD clinic patients and a matched control group using the complement fixation test.

A collaborative study with the U.S. Navy also was undertaken to evaluate NGU. Males entering an infirmary with urethritis and having no evidence of a gonococcal infection were considered NGU patients. Each patient completed a questionnaire relating to epidemiology and clinical disease. A pertinent physical examination was done and an acute blood specimen drawn. Each patient had urethral specimens examined for Chlamydia trachomatis, Trichomonas vaginalis, herpesvirus hominis, cytomegalovirus (CMV), and mycoplasma. Each patient was given a standard regimen of tetracycline and asked to return in two weeks for a follow-up evaluation and convalescent blood specimen.

Major Findings

The pilot study done at the VD clinic revealed that NGU was a significant problem in this clinic, accounting for about 75% of all the urethritis seen in males. In the series of 26 NGU patients, C. trachomatis was isolated from the urethra of 11 (42%). Trichomonas vaginalis and herpesvirus also were found in a small percentage of cases. Epidemiologic characteristics of NGU patients were compared with those of patients with gonorrhea and some significant differences were observed. NGU patients tended to be older, caucasian, have a longer duration of symptoms before attending the VD clinic, and engage in sexual intercourse despite symptoms much more frequently. Serologic studies indicated that significantly more Chlamydia and herpesvirus infections had occurred in the VD clinic population than in a matched control group.

The collaborative study with the Navy was recently completed except analysis of data. A total of 128 men were studied. This group uded both patients and controls and it is not yet known which specimens obtained from patients and which from controls. In the entire group ıen there were 34 C. trachomatis isolations, 4 herpesvirus isolations, ichomonas vaginalis isolate and no CMV virus isolations. The prevalence complement fixing antibody to Chlamydia, herpesvirus and CMV also was rmined. The clinical, laboratory, and epidemiologic information on e patients is currently being programmed for computer analysis.

ficance to Biomedical Research and the Program of the Institute

The sexually transmitted diseases are currently a major public health lem. Recent investigations have revealed that non-gonococcal urethritis) has become one of the most commonly encountered forms of urethritis in males. Relatively little is known about NGU in the United States use this disease is not included in the venereal disease statistics of Public Health Service. Consequently, good data on incidence, epidemiol- and clinical manifestations of this disease are not available. A ificant proportion of NGU has recently been shown to be due to C. nomatis.

Extensive investigations on the relationship of C. trachomatis to n disease have not been done, although these organisms are known to be onsible for causing trachoma, inclusion conjunctivitis and lympho- uloma venereum. The recent developments of cell culture techniques serology have made culture and serologic diagnosis of Chlamydia ctions both reliable and sensitive. These developments have made the ematic evaluation of C. trachomatis infections in human populations ible.

osed Course of the Project

Although a significant percent of NGU has recently been shown to be to C. trachomatis, there remains a relatively large number of patients NGU for whom the etiology is unknown. These studies will be extended valuate the influence of bacterial urethral flora in this form of ıritis. This will be accomplished by comparing urethral bacterial ates in Chlamydia-positive and Chlamydia-negative NGU patients.

Acute epididymitis is another area where C. trachomatis has recently implicated as a possible etiologic agent. Acute epididymitis is of ary interest because of its ability to incapacitate healthy young men. llaboration with the Urology department at Tripler Army hospital a ematic evaluation of the role of C. trachomatis in epididymitis will ıdertaken.

Current efforts are being directed at developing techniques to serotype organisms belonging to the C. trachomatis group and the capability of performing serotype-specific serology. These techniques will be utilized to examine the prevalence of antibody to various C. trachomatis serotypes in South Pacific island populations where information on these agents is non-existent.

Publications:

Kuberski, T., Perry, S., Ohye, R., and Wiebenga, N.: Non-gonococcal urethritis in Hawaii. Hawaii Med. J. 1976 (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00151-01 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

STUDIES ON THE ECOLOGY OF ENCEPHALOMYOCARDITIS VIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. B. TESH	STAFF MEMBER, PACIFIC RESEARCH SECTION, LPD, NIAID
OTHER:	G. D. WALLACE	STAFF MEMBER, PACIFIC RESEARCH SECTION, LPD, NIAID
	L. ROSEN	HEAD, PACIFIC RESEARCH SECTION, LPD, NIAID

COOPERATING UNITS (if any)
VECTOR CONTROL BRANCH, HAWAII STATE HEALTH DEPARTMENT; VETERINARY LABORATORY, HAWAII STATE DEPT. OF AGRICULTURE; PUBLIC HEALTH LABORATORY SERVICE, NATIONAL HEALTH SERVICE, UNITED KINGDOM; CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

LAB/BRANCH
LABORATORY OF PARASITIC DISEASES

SECTION
PACIFIC RESEARCH SECTION

INSTITUTE AND LOCATION
NIAID, NIH, HONOLULU, HAWAII

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

SERA FROM HAWAIIAN WILD AND DOMESTIC ANIMALS WERE EXAMINED BY PLAQUE REDUCTION NEUTRALIZATION METHOD FOR EVIDENCE OF ENCEPHALOMYOCARDITIS VIRUS NEUTRALIZING ANTIBODIES. OF THE ANIMALS TESTED, THE HIGHEST PREVALENCE OF INFECTION WAS FOUND IN RATTUS RATTUS. INFECTION RATES VARIED WIDELY AMONG RATS FROM DIFFERENT LOCALITIES.

NEUTRALIZATION TESTS PERFORMED ON HUMAN SERA FROM VARIOUS REGIONS OF THE WORLD INDICATED A HIGH PREVALENCE OF EMC VIRUS ANTIBODIES AMONG CERTAIN PACIFIC ISLAND POPULATIONS. AGE-SPECIFIC ANTIBODY RATES AMONG THESE GROUPS SUGGEST THAT INFECTION IS ACQUIRED IN EARLY CHILDHOOD. EXAMINATION OF SERA FROM 400 JUVENILE DIABETICS AND CONTROL CHILDREN FAILED TO DEMONSTRATE AN ASSOCIATION BETWEEN JUVENILE DIABETES AND EMC VIRUS INFECTION.

THESE STUDIES ARE DESIGNED TO LEARN MORE ABOUT THE ECOLOGY OF EMC VIRUS AND ITS DISEASE POTENTIAL IN MAN.

PHS-6040
(12-75)

PROJECT DESCRIPTION:

OBJECTIVES

1. TO STUDY THE EPIDEMIOLOGY OF ENCEPHALOMYOCARDITIS (EMC) VIRUS INFECTION AMONG RATS AND OTHER WILD AND DOMESTIC ANIMALS IN HAWAII.

2. TO STUDY THE PREVALENCE AND CLINICAL MANIFESTATIONS OF EMC VIRUS INFECTION AMONG SELECTED HUMAN POPULATIONS.

METHODS EMPLOYED

EXAMINATION OF HUMAN AND ANIMAL SERA FOR EMC ANTIBODIES WAS CARRIED OUT BY PLAQUE REDUCTION NEUTRALIZATION METHOD IN MICROPLATE CULTURES OF VERO CELLS.

MAJOR FINDINGS

A. PREVALENCE OF EMC VIRUS NEUTRALIZING ANTIBODIES AMONG HAWAIIAN WILD AND DOMESTIC ANIMALS.

SERA FROM RATS, MONGOOSES, CATS, BOVINES AND SWINE FROM SEVERAL DIFFERENT HAWAIIAN ISLANDS WERE EXAMINED BY PLAQUE REDUCTION NEUTRALIZATION TEST FOR ANTIBODIES AGAINST EMC VIRUS. OF THE ANIMAL SPECIES EXAMINED, THE HIGHEST INFECTION RATES WERE FOUND IN RATTUS RATTUS; HOWEVER, CONSIDERABLE VARIATION WAS NOTED IN THE PREVALENCE OF ANTIBODIES AMONG RATS FROM DIFFERENT ISLANDS. FOR EXAMPLE, EMC ANTIBODY RATES AMONG R. RATTUS FROM THE ISLAND OF OAHU WERE 8.2%, WHILE THOSE OF THE SAME SPECIES COLLECTED ON THE ISLAND OF MOLOKAI WERE 85.9%. THESE RESULTS SUGGEST THAT MOLOKAI IS AN AREA OF HYPER-ENDEMIC EMC VIRUS ACTIVITY AND FUTURE FIELD STUDIES ON THE ECOLOGY OF THE VIRUS ARE PLANNED THERE.

EMC VIRUS INFECTION RATES AMONG CATS WERE LOW (3%) WHILE THOSE OF MONGOOSES WERE INTERMEDIATE (36%). THE EXPLANATION FOR THESE SPECIES DIFFERENCES ARE UNKNOWN AT PRESENT BUT INDICATE THAT THE VIRUS HAS A WIDE NATURAL RANGE. SEROLOGIC STUDIES OF PORCINE AND BOVINE SERA WERE COMPLICATED BY THE PRESENCE OF APPARENT NON-SPECIFIC NEUTRALIZING SUBSTANCES IN THE SERA OF MANY OF THESE ANIMALS.

B. PREVALENCE OF EMC VIRUS INFECTION AMONG VARIOUS HUMAN POPULATIONS

IN ORDER TO LEARN MORE ABOUT THE EPIDEMIOLOGY OF EMC VIRUS INFECTION IN MAN, APPROXIMATELY 1,500 HUMAN SERA FROM VARIOUS LOCATIONS IN THE WORLD WERE SCREENED BY PLAQUE REDUCTION NEUTRALIZATION TEST FOR EMC ANTIBODIES. A

MARKED VARIATION IN THE PREVALENCE OF ANTIBODIES WAS OBSERVED AMONG DIFFERENT POPULATIONS. FOR EXAMPLE, EMC ANTIBODY RATES AMONG RESIDENTS OF THE UNITED STATES, AUSTRALIA AND THE UNITED KINGDOM WERE RELATIVELY LOW, RANGING FROM 1 TO 7%, WHILE THOSE OF POPULATION SAMPLES FROM SEVERAL PACIFIC ISLANDS (AMERICAN SAMOA, NEW HEBRIDES AND NEW GUINEA) VARIED FROM 27 TO 51%. THESE DIFFERENCES MAY BE RELATED TO THE LOWER LEVELS OF SANITATION AND ABUNDANCE OF RATS ON THE LATTER ISLANDS. ALTHOUGH THE CLINICAL MANIFESTATIONS OF EMC VIRUS INFECTION IN MAN ARE POORLY UNDERSTOOD, OUR SEROLOGIC RESULTS INDICATE THAT HUMAN INFECTION WITH THIS VIRUS IS FAIRLY COMMON IN SOME AREAS OF THE WORLD. EXAMINATION OF SERA FROM PERSONS OF DIFFERENT AGE GROUPS IN COMMUNITIES WHERE THE PREVALENCE OF EMC VIRUS ANTIBODIES IS HIGH SUGGEST THAT INFECTION OCCURS IN EARLY CHILDHOOD, ALTHOUGH THE MODE AND SOURCE OF INFECTION ARE UNKNOWN.

C. EXAMINATION OF SERA FROM JUVENILE DIABETICS FOR EVIDENCE OF EMC VIRUS INFECTION.

BECAUSE OF RECENT FINDINGS THAT EMC VIRUS INFECTION PRODUCES A DIABETES MELLITUS-LIKE DISEASE IN CERTAIN STRAINS OF INBRED MICE, 400 SERA FROM JUVENILE DIABETICS AND AGE-MATCHED CONTROL CHILDREN FROM THE UNITED KINGDOM WERE EXAMINED FOR EMC VIRUS NEUTRALIZING ANTIBODIES. ONE OF 200 DIABETICS AND 3 OF 200 CONTROL CHILDREN HAD EMC ANTIBODIES, THUS THERE WAS NO EVIDENCE FOUND OF AN ASSOCIATION BETWEEN JUVENILE DIABETES AND EMC VIRUS INFECTION IN THIS POPULATION.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

ENCEPHALOMYOCARDITIS VIRUS IS A MEMBER OF THE ENTEROVIRUS GROUP. HOWEVER, UNLIKE MOST OF THE OTHER KNOWN ENTEROVIRUSES, WHICH ARE QUITE HOST-SPECIFIC, EMC HAS A WIDE NATURAL HOST RANGE. ALTHOUGH THE VIRUS HAS MOST OFTEN BEEN ASSOCIATED WITH RODENTS, IT HAS ALSO BEEN RECOVERED FROM A NUMBER OF DIFFERENT PRIMATE SPECIES, INCLUDING MAN. VERY LITTLE IS KNOWN ABOUT ITS ECOLOGY OR HUMAN DISEASE POTENTIAL. SPORADIC REPORTS IN THE MEDICAL LITERATURE HAVE ASSOCIATED EMC VIRUS INFECTION WITH HUMAN CASES OF ENCEPHALITIS AND POLIO-LIKE ILLNESSES. IN ADDITION, SEVERAL OUTBREAKS OF EMC VIRUS INFECTION WITH HIGH MORTALITY HAVE BEEN REPORTED AMONG SWINE HERDS IN THE UNITED STATES, PANAMA AND AUSTRALIA. IN NATURALLY AND EXPERIMENTALLY INFECTED PIGS, THE VIRUS PRODUCES AN ACUTE, OFTEN FATAL MYOCARDITIS. RECENTLY, EMC VIRUS INFECTION HAS BEEN SHOWN TO PRODUCE A DIABETES MELLITUS-LIKE DISEASE IN CERTAIN STRAINS OF INBRED MICE.

IT HAS BEEN SUGGESTED, THAT RATS ARE THE NATURAL RESERVOIR OF EMC VIRUS. RECENT STUDIES IN HAWAII INDICATE THAT THE VIRUS IS ENDEMIC AMONG WILD RAT AND MONGOOSE POPULATIONS ON SEVERAL OF THE ISLANDS. THIS WORK IS DESIGNED TO LEARN MORE ABOUT THE ECOLOGY OF EMC VIRUS IN THESE ANIMAL POPULATIONS. A HIGH PREVALENCE OF EMC VIRUS NEUTRALIZING ANTIBODIES HAS BEEN FOUND AMONG

HUMAN POPULATIONS LIVING ON SEVERAL PACIFIC ISLANDS, INDICATING THAT HUMAN INFECTION WITH THE VIRUS IS COMMON IN THESE AREAS. IT IS HOPED THAT THESE SEROLOGIC STUDIES WILL YIELD NEW INFORMATION ON THE EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS OF EMC VIRUS INFECTION IN MAN.

PROPOSED COURSE OF THE PROJECT

FUTURE FIELD STUDIES ARE PLANNED IN HAWAII IN AN AREA WHERE EMC VIRUS APPEARS TO BE ENDEMIC. THESE WILL INCLUDE AN ATTEMPT TO ISOLATE THE VIRUS FROM WILD RATS. IF AN ORIGINAL ISOLATE CAN BE OBTAINED, EXPERIMENTAL STUDIES IN RATS ARE PLANNED TO DETERMINE THE EFFECT OF EMC VIRUS INFECTION AND THE LENGTH OF VIRUS EXCRETION IN RODENT FECES. ADDITIONAL SEROLOGIC STUDIES ARE PLANNED IN SELECTED HUMAN PATIENTS WITH DISEASES SUCH AS ENCEPHALITIS AND MYOCARDITIS IN HOPES OF LEARNING MORE ABOUT THE DISEASE POTENTIAL OF EMC VIRUS INFECTION IN MAN.

PUBLICATIONS: NONE

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00152-01 LPD

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on congenital transmission of influenza virus in humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. D. Wallace, Staff Member, Pacific Research Section, LPD, NIAID
OTHER: T. T. Kuberski, Staff Member, Pacific Research Section, LPD, NIAID

COOPERATING UNITS (if any)

Kaiser Medical Center, Honolulu, Hawaii

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Pacific Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Honolulu, Hawaii

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Since definitive information on the transplancental transmission of influenza virus is lacking, a prospective investigation relating to this subject was initiated during a recent influenza epidemic in Hawaii. Appropriate specimens for laboratory confirmation of influenza were obtained from 15 pregnant women with an influenza-like illness. Infection with influenza virus (A/Hawaii/12/75[H3N2], similar to A/Victoria) was confirmed in 8. Thus far 2 of these women have delivered, and umbilical cord blood and placenta have been collected from each. Results of attempts to isolate virus from the placenta are pending. Tests for specific IgM type influenza antibody in cord serum of each were negative.

PHS-6040
(12-75)

Project Description:

Objectives

To determine if congenital transmission of influenza virus occurs in humans.

Methods Employed

During a recent influenza epidemic in Hawaii, a prospective study was initiated to provide information on the question of transplancental transmission of influenza virus. Paired blood specimens and oropharyngeal swabs were collected from pregnant patients seen at Kaiser Medical Center with signs and symptoms suggestive of influenza. Umbilical cord blood and placental tissues are then collected during delivery from patients with confirmed influenza. Also, a blood specimen and an oropharyngeal swab are collected from the neonate. Serum collected during the acute phase of influenza and placental tissue obtained at parturition are tested for virus by standard techniques. The presence of specific IgM type antibody in the cord serum is tested for by a standard hemagglutination inhibition test, following treatment with 2'-mercaptoethanol (2-ME).

Major Findings

Specimens were submitted from 15 pregnant women with influenza-like disease. Of these, infection with influenza virus was confirmed in 8 by the demonstration of antibody titer rise to A/Hawaii/12/75 antigen. Virus similar to A/Hawaii has been isolated from oropharyngeal swabs of 3 of the serologically confirmed cases. Work on the isolation of virus from the other 5 confirmed cases is still in progress. To date, cord blood and placenta have been received from 3 confirmed cases. Tests on cord sera of 2 have been completed. Influenza antibody was not detected in one (an unusual finding since antibody was present in the mother before delivery) and 2-ME treatment of the other failed to reduce the antibody titer.

Significance to Biomedical Research and the Program of the Institute

Published information on congenital influenza is conflicting. Based on data from retrospective studies following influenza epidemics, some investigators have suggested that influenza is transmitted congenitally and may produce pathology in the fetus. However, transplancental transmission of influenza virus in humans has been proven virologically in only a few severe cases in which the mother and fetus succumbed to influenza.

In one report on a prospective serologic study, the authors concluded that the virus was not transmitted congenitally. However, data from another prospective serologic study indicated that virus was transmitted to the fetus frequently in mothers with confirmed influenza. Of particular interest are retrospective statistical studies relating increased risk of leukemia in children to birth during certain epidemics of influenza. Definitive information on transplancental transmission of influenza virus is obviously needed.

Proposed Course of the Project

It is anticipated that appropriate specimens will be otained at parturition from the remaining patients in the study. In the vent of another influenza epidemic in Hawaii, efforts will be made to study a larger number of patients.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00106-04 LPD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on humoral immunity of malaria and other parasitic diseases.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Kendall G. Powers
Other Investigators: L.H. Miller and M.N. Lunde

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
None		

SUMMARY OF WORK (200 words or less - underline keywords)

Project terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00109-04 LPD

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cell-mediated immunity in malaria.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: David J. Wyler

Other Investigators: L.H. Miller and J.I. Gallin(LCI)

COOPERATING UNITS (if any)
Laboratory of Clinical Investigation, NIAID; Medical Research Council Laboratories, Fajara, The Gambia (West Africa); Southern Research Laboratories, Birmingham, Alabama.

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, Bethesda,Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
None		

SUMMARY OF WORK (200 words or less - underline keywords)

Because the principal investigator will be away from the NIH for one year (June 30, 1975-July 1, 1976) this project will be temporarily curtailed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00112-02 LPD

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Host controlled modifications in malaria parasites.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Richard Carter

Other Investigators: L.H. Miller

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

LABORATORY OF IMMUNOLOGY

Summary I

Project No.

Z01 AI 00030-08 - Antigen Recognition and Activation of Immunocompetent Cells 9

Z01 AI 00033-08 - Lymphocyte Interactions, Receptors and Functions 14

Z01 AI 00034-04 - Differentiation and Activation of Mouse T and. B Lymphocytes 19

Z01 AI 00035-03 - Specificity in Immune Response 24

Z01 AI 00036-11 - Regulation of Gene Expression and Lymphoid Differentiation 30

Z01 AI 00037-09 - Immunogenetics of Mouse Immunoglobulins and Genetic Control of Antibody Response 35

Z01 AI 00038-04 - Structural and Activity Studies on Immunologically Important Cells and Proteins 40

Z01 AI 00040-02 - Genetic Control of Immunocompetent Cell Interactions 44

Z01 AI 00147-01 - The Mechanism of Activation of Thymus-Derived Lymphocytes 49

Z01 AI 00148-01 - A Receptor for Complement in Tissues in Immunopathological conditions 52

Z01 AI 00039-01 - Terminated Project - Tumor Immunology in Inbred Guinea Pigs 54

PHS-NIH
Summary statement
Office of the Chief
Laboratory of Immunology
July 1, 1975 through June 30, 1976

Genes Controlling Cross-Reactive Idiotypes Found on Anti-Levan Antibodies are Linked to IgC_H Genes

The genetic basis of antibody diversity is one of the most challenging problems of both immunobiology and genetics. A major clarification of several aspects of this problem has been obtained by the identification of a genetic region, the IgC_H region, in which the structural genes for the constant regions of immunoglobulin (Ig) heavy (H) chains are found. Studies of idiotypic determinants expressed on the variable regions of certain antigen-binding myeloma proteins have demonstrated that these idiotypes may also be identified on normal antibodies in some, but not all, mouse strains. Genes controlling the expression of these idiotypes have been shown previously, both by work carried out in the Laboratory of Immunology and elsewhere, to be closely linked to the IgC_H genes. This year, studies of a gene (or complex of linked genes) which controls expression of a family of distinct cross reactive idiotypic determinants found both on levan-binding myeloma proteins and antibodies indicates that this gene is also closely linked to the IgC_H region. Initial recombination studies indicate the separation of IgC_H genes and cross-reactive idiotype genes can be obtained at a frequency of $\sim$ 1%. Continued studies of these recombinants should yield evidence concerning the genetic basis of the cross-reacting idiotypes. In turn, this should be most important in the understanding of the genetic organization of the chromosomal region controlling immunoglobulin structure and diversity (Lieberman and Humphrey, LI/NIAID; Potter, LCBGY/NCI).

Differentiation Sequence of Mouse B Lymphocytes

It is increasingly apparent that the two principal classes of lymphocytes, the thmus-dependent (T) lymphocytes and the thymus-independent (B) lymphocytes, may each be subdivided into populations of distinct cells based both on functional properties and membrane markers. The study of B lymphocytes has been markedly aided by detailed observations of the functions and surface markers of B lymphocytes obtained from CBA/N mice. These animals have an X-linked defect in immune function characterized by a failure to respond to certain thymus (T)-independent antigens and by an apparent B lymphocyte maturational arrest. Previous studies had established that CBA/N B lymphocytes have an abnormal density of membrane immunoglobulin (Ig), an elevated ratio of IgM to IgD on the cell membrane, a paucity of complement receptor lymphocytes (CRL) and an absence of cells capable of stimulating Mls determined mixed lymphocyte responses. By detailed comparison of adult CBA/N B lymphocytes to lymphocytes obtained from developing mice of various ages, it has been shown that the set of characteristics noted in adult CBA/N mice describes a subset of B lymphocytes normally present in developing mice, and probably at lower frequency in adult mice. Moreover, this work suggests that

functional differences exist between the B lymphocyte subsets and that responsiveness to certain T-independent antigens, such as 2,4-dinitrophenyl-(DNP)-Ficoll and type III pneumococcal polysaccharide (SIII) depends upon the action of mature B lymphocytes while less mature B cells can respond to other antigens such as trinitrophenyl (TNP)-brucella abortus. These studies should provide major insights into the development of the B cell system and into the relative immunocompetence of the immature individual (Mosier, Mond, Zitron and Paul, LI/NIAID; Sher, Ahmed and Zaldivar, NMRI).

Genetic Restriction of T Lymphocyte Activation by Antigen

Thymus-dependent (T) lymphocytes from primed inbred or F_1 hybrid guinea pigs can be efficiently activated only if antigen is presented to them on either a macrophage or a related cell found in peritoneal exudates. This activation depends upon the antigen-pulsed peritoneal exudate cell (PEC) and the primed T lymphocyte being derived from syngeneic or semi-syngeneic donors. Genetic studies indicate that the critical genes which must be held in common by the interacting cells are found in the I-region of the guinea pig major histocompatibility complex (MHC). One explanation of this result is that efficient cellular interactions require similarity at an MHC encoded cellular interaction (CI) gene. An alternative possibility is that T lymphocytes normally recognize a complex antigenic determinant (CAD) consisting of the nominal antigen and the product of an I-region gene. If this were so, T lymphocytes from primed (2x13)F_1 guinea pigs should be divisible into two distinct antigen-responsive sets, one which recognizes a CAD consisting of antigen and strain 2 I-region gene products and the other which recognizes a CAD involving antigen and strain 13 I-region gene products. This was shown to be true for ovalbumin (OVA) and purified protein derivative of tuberculin (PPD)-responsive T cells from primed (2x13)F_1 donors. Independent clones of F_1 T lymphocytes responsive to OVA-pulsed 2 PECs and to OVA-pulsed 13 PECs were demonstrated by both positive and negative selection techniques; similar results were obtained for PPD-responsive cells. These experiments strongly suggest that T lymphocyte antigen-recognition is based on the formation of complexes between antigen and cell membrane structures coded for in the I-region of the MHC and that receptors of T lymphocytes are principally specific for such complex antigenic determinants (Paul, Shevach and Thomas, LI/NIAID; Rosenthal LCI/NIAID).

In Vitro Primary T Lymphocyte Proliferative Responses; Evidence for Importance of I-region Gene Products in T Lymphocyte Activation.

One major limiting feature in the study of T lymphocyte proliferative response to conventional antigens is that such experiments have heretofore been feasible only for cells obtained from donors primed in vivo. This has prevented the in vitro study of important events in the priming process and particularly has impeded the study of the role of MHC gene products in lymphocyte activation. In experiments carried out this year, a simple in vitro primary response system was developed. Lymph node lymphocytes from non-immune guinea pigs were cultured with antigen-pulsed peritoneal exudate cells (PECs) for one week, harvested, washed and recultured with fresh PECs which were either pulsed with antigen or not. When reexposed to the same antigen-pulsed PECs as used in the primary a marked proliferative response ensued. This response depended not only on the specificity of the antigen but on the origin of the PECs. Thus, if strain 2 lymph node (LN) cells were initially cultured with OVA-pulsed 2 PECs,

they could be restimulated by OVA-pulsed 2 PECs but not by OVA-pulsed 13 PECs. Furthermore, if LN cells from non-primed (2x13)F_1 donors were initially cultured with OVA-pulsed 2 PECs they could be subsequently stimulated by OVA-pulsed 2 but not 13 PECs. These results have essentially the same implications as those described in the previous section. However, this system allows the exploration of the possibility of priming T lymphocytes with antigen-pulsed allogeneic PECs. Although certain technical difficulties remain to be solved before that experiment can be performed, it has been shown that T lymphocytes from strain 2 guinea pigs can be sensitized by strain 13 PECs which have been treated with anti-13 Ia sera, suggestive that 2 lymphocytes can potentially respond to complex antigenic determinants involving 13 I-region antigens and that the construction of complex antigenic determinants is not a function limited to I-region gene products of "self" (Thomas and Shevach, LI/NIAID).

Specific Inhibition of Mouse T Lymphocyte Proliferative Responses with Anti-Ia Antisera

Until recently, the direct evaluation of antigen-stimulated mouse T lymphocyte responses was difficult because no readily reproducible in vitro assay existed. In last year's report, a sensitive and reliable technique was described based on the antigen-stimulated proliferation of enriched populations of T lymphocytes obtained from peritoneal exudates of primed mice. This year, this assay has been used to study several aspects of T lymphocyte function and particularly of the genetic control of T lymphocyte activation. In 8 separate systems in which it was known that antibody responses were controlled by MHC-linked immune response (Ir) genes, the only strains which developed antigen-responsive T lymphocytes were those which produced antibodies upon immunization. This indicates that identical, or closely linked, Ir genes control T cell proliferative responses and antibody responses. The importance of this finding is that in at least one of these genetically controlled systems, data has been presented by others which suggests that some Ir genes function principally in B lymphocytes. Our study indicates that these Ir genes also control a response to which B lymphocytes make no contribution and thus such genes cannot be exclusively expressed in B lymphocytes.

In addition, it was shown that antisera directed at products of the I-region of the mouse MHC inhibited T lymphocyte activation while antisera directed at products of the K and/or D regions of the MHC had little or no effect. In systems controlled by specific Ir genes, anti-I sera only inhibited the response if they were directed against products of the particular I sub-region in which the Ir gene was mapped. Furthermore, in F_1's of responders by non-responders, anti-Ia sera blocked only if directed at the product of the allele of the I gene linked to the Ir gene controlling the response. This result indicates a very close molecular or steric relationship between the products of genes controlling Ia antigens and of Ir genes. Indeed, the simplest explanation is that Ia antigens and Ir gene products are on the same molecule. (R. Schwartz and Paul, LI/NIAID, Sachs, TB/NCI, David, Washington Univ., Dorf and Benacerraf, Harvard Medical School).

Serological and Chemical Analysis of Guinea Pig Ia Antigens

The major histocompatibility complex (MHC) of guinea pigs encodes two

types of membrane antigenic determinants, the Ia antigens, which are principally expressed on lymphoid cells and which are comparable to the mouse Ia antigens, and the B and S antigens, which have a wide tissue distribution and which appear comparable to the K and D antigens of mice. Serological studies using a variety of sera produced by cross immunization of inbred and of outbred guinea pigs have lead to the identification of 7 Ia antigens, designated Ia.1-7. Inbred strain 2 guinea pigs possess Ia.2,4,5 and 6 while strain 13 guinea pigs possess Ia.1,3,5,6 and 7. Chemical studies of these antigens indicate the existence of three families of Ia molecules, each family with a distinctive structure. Ia.4 and 5 (in strain 2 guinea pigs) are clearly on a molecule composed of two glycoproteins cross-linked by disulfide bands. The two polypeptide chains are 33,000 and 25,000 daltons, respectively and the disulfide-bonded unit is 58,000 daltons. Ia.2 in strain 2 guinea pigs is also associated with two polypeptide chains of 33,000 and 25,000 daltons, respectively. In this instance, however, the chains are not disulfide-linked although they are probably jointed by noncovalent bonds. Finally, Ia.6 in strain 2 guinea pigs appears to be associated with a single chain of $\sim$ 25,000 daltons. A comparable group of 3 distinct types of Ia antigens exists in strain 13 guinea pigs. No evidence of association of these Ia antigens with β_2 microglobulin has been obtained. The functional significance of the two chain structure of Ia antigens and of the structurally distinct types of Ia antigens are now under investigation (Shevach, B. Schwartz and Paul, LI/NIAID; Geczy and deWeck, Berne, Switzerland).

Preliminary Amino Acid Sequence of N-Terminal Region of Guinea Pig B.1 Antigen

The guinea pig major histocompatibility complex (MHC) codes for two principal types of membrane glycoprotein antigens. ONe type, the B and S antigens, are analagous to the mouse D and K antigens and to the human HLA-A and HLA-B antigens. These antigens play an important role in transplantation immunity and in the interaction of cytotoxic T lymphocytes with target cells. Preliminary sequence data of mouse and human antigens indicate substantial homology. In order to further explore the evolutionary relationship of these antigens and to try to identify structural reasons for their unique functions, the N-terminal region of the B.1 allele of guinea pig B antigen was sequenced by radiochemical procedures. Lymph node cells from strain 2 guinea pigs were cultured for 4 hours with a mixture of 10 tritiated amino acids of high specific activity. The cells were then lysed with NP-40 and membrane glycoproteins were partially purified by passage over lens culinaris lectin columns. The radioactive B.1 antigen was purified by precipitation with anti-B.1 serum and electrophoresis on SDS-polyacrylamide gels. The antigen was then subjected to sequential Edman degradation in the automated sequencer and radioactive amino acids identified by high pressure liquid chromatography and thin layer chromatography. In the first 15 positions, 9 residues were identified. They are: 3-histidine; 5-leucine; 6-arginine; 7-tyrosine; 8-phenylalanine; 9-tyrosine; 11-alanine, 12-valine; 15-proline. Each of these amino acids has already been found in a mouse K or D or human HLA antigen at the same position. Furthermore, 6 of the identified positions are identical in human, mouse and guinea pig indicating a high order of evolutionary conservation among these biologically important and highly polymorphic glycoproteins. These studies are now being extended to the remainder

of the allelic forms of the B antigens and to S. In addition, preliminary Ia sequencing is in progress (B. Schwartz, Waxdal, Shevach and Paul, LI/NIAID; MacMillan and Hood, California Institute of Technology).

Critical Role of Ia Antigens in Immunogenicity of Tumor Associated Transplantation Antigen(s) (TATA) of Guinea Pig L_2C Leukemia

Experiments performed in several laboratories, including the Laboratory of Immunology, indicate a critical role for Ia-like antigens in the initiation of immune responses to conventional antigens and to membrane alloantigens. On the other hand, D and K antigens of the mouse and B antigens of the guinea pig appear important as targets of effector cells in transplantation immunity. The availability of a family of L_2C leukemic lines, all descended from the same initial leukemia of strain 2 guinea pigs, but varying in their surface histocompatibility antigens, allowed a direct test of the role of Ia antigens in tumor immunity. Five separate L_2C lines were obtained, each of which shared a chromosomal marker and an idiotypic determinant on membrane associated IgM. One of these five lines lacked detectable Ia antigens while the other four bore the Ia antigens characteristic of strain 2 donors. All the tumors possessed the B.1 histocompatibility antigen. Immunization-protection tests indicated that each of the four Ia positive L_2C tunors initiated a protective immune response, while the Ia negative L_2C tumor was not immunogenic. However, strain 2 guinea pigs preimmunized with any of the Ia positive L_2C lines were protected against lethal challenge with the Ia negative, non-immunogenic L_2C line. This indicates that L_2C lines possess a common TATA but that Ia antigens play a critical role in the initiation of immune responses to this antigen just as they are important in the regulation of immune responses to conventional antigens (Forni, Shevach and Green, LI/NIAID).

The Receptor for C3B in the Human Renal Glomerulus is Important in In Vivo Complement Deposition.

Many immunologically mediated renal diseases are characterized by the deposition of complexes of antigen, antibody and complement (C) on epithelial cells of the renal glomerulus. In turn, these complexes initiate the inflammatory response which leads to local tissue damage and to functional impairment. Last year, it was shown that a receptor for the third component of C existed in the renal glomerulus of humans and that the receptor appeared to be specific for an activated fragment of this component (3b). This year it was demonstrated that the receptor was located on the visceral epithelial cell, which is the location in which antigen-antibody-C complexes are found in many renal diseases. Furthermore, when kidney biopsies were examined from patients with various forms of renal disease, an inverse relation existed between the existence of C, deposited *in vivo*, in the glomerulus and the ability of antigen-antibody-C complexes to be bound *in vitro*. This is consistent with the concept that in the pathogeneses of immune complex renal disease complexes containing complement are bound by the C3 receptors. These receptors are then saturated and cannot bind freshly added antigen-antibody-complement complexes *in vitro*. These observations should form the basis of the rational understanding of the mechanism by which complement is deposited in the glomerulus, *in vivo*, and may allow the development of

strategies to limit or reverse such deposition. (Green, LI/NIAID; Frank, LCI/NIAID; Gelfand, LI/NIAID and Georgetown Medical Center; Shin and Nagle, Univ. of Md.).

Administrative, Organizational, Other Changes

Laboratory of Immunology scientists continued to make contributions of substantial scientific importance in the past year. This is shown both by the increasing number of scientific publications from the laboratory and by the participation of Laboratory staff in major national and international scientific meetings. During the past year, three research associates, Drs. Philip Cohen, Andrew Drexler and Fred Finkelman completed their training in the Laboratory as did Dr. Guido Forni, a Visiting Fellow. In addition, Dr. Jaroslav Rejnek completed a six month stay as a Visiting Scientist during this year. Each of these individuals have made important contributions to the Laboratory program. Three new associates, Drs. Eliot Goldings, Theodore Konen and James Mond, joined the laboratory this year as did Drs. Chau-Chun Chien, Helen Hu, Tsuyoshi Sakane and Akihiko Yano, all of whom are Visiting Fellows. Drs. Hans Potgieter, Donna Sieckman and David Thomas entered the Laboratory as guest workers.

Honors, Awards, and Scientific Recognition

Laboratory scientists play important roles in the national and international scientific community. Many serve on editorial boards of important journals. Dr. Paul is section editor for cellular immunology for the Journal of Immunology and Drs. Green, Mage and Mosier are associate editors of this journal. Dr. Mage is a member of the editorial board of Federation Proceedings and Drs. Green and Inman are members of the Board of Reviewers of this journal. Drs. Mage and Paul are members of the editorial board of the Journal of Immunologic Methods and Drs. Green and Paul are referee editors of the Proceedings of the Society for Experimental Biology and Medicine. In addition, Dr. Paul is an associate editor of the Journal of Experimental Medicine, Immunogenetics, the Scandanavian Journal of Immunology and Immunologic Communications. Dr. Green is a member of the editorial board of Clinical Immunology and Immunopathology, and Ms. Lieberman is an advisory editor of Immunochemistry. Dr. Green is editing a book on Mechanisms in Tumor Immunology for John Wiley and Sons and Dr. Shevach is a member of the editorial advisory board of the FASEB Handbook on Inbred Strains of Laboratory Animals.

Dr. Paul chaired sessions at the Gordon Conference on Molecular Pathology, Tilton, N.H., at the Brook Lodge Conference on the Role of the Histocompatibility Gene Complex in Immune Responses and at the Belmont House Conference on Development of Host Defenses. He gave major presentations at the Southeast Regional Meeting of the American Rheumatism Association, at the Midwinter Conference of Immunologists at Asilomar, California and at the Cold Spring Harbor Symposium on Origins of Lymphocyte Diversity. In addition, he was a lecturer in the American Association of Immunologists Survey Course in Immunology, in the Cold Spring Harbor course on Immunogenetics in Relation to Experimental Tumor Immunology and at the NATO Advanced Study Institute on Molecular and Cellular Aspects of Allergy at Falcon Lake, Manitoba. Dr. Paul was a Phillips Distinguished Visitor at Haverford College and was elected to the council of the American Society of Clinical Investigation. He serves as a member of the Travel Committee of the American Association of Immunologists and of the Transplantation and Immunology Advisory Committee of NIAID.

Dr. Mage received a USPHS Superior Service Award; she was an invited speaker at the Cold Spring Harbor Conference on Origins of Lymphocyte Diversity and the VIIth Homogeneous Immunoglobulin Workshop. She is councilor of the Washington, D.C. chapter of Sigma Xi and is a member of the American Association of Immunologists Committee on the Status of Women and Minorities. She is a member of the Allergy and Immunology Study Section of the Division of Research Grants, NIH.

Dr. Green received a USPHS Superior Service Award; he presented an invited lecture at the Brook Lodge Conference on the Role of the Histocompatibility Gene Complex in Immune Responses and served as a member of the program committee of the American Association of Immunologists.

Dr. Shevach presented invited lectures at the Gordon Conference on Molecular Pathology, Tilton, N.H. and at the Brook Lodge Conference on the

Role of the Histocompatibility Gene Complex in Immune Responses. He was appointed to the Allergy and Immunology Study Section of the Division of Research Grants, NIH. Ms. Lieberman was an invited speaker at the VIIth Homogeneous Immunoglobulin Workshop and served as a member of the program committee of the American Association of Immunologists. Dr. Mosier gave invited presentations at the First International Symposium on Bone Marrow Transplantation in New York, at the Mid-West Immunology Conference in Chicago, at the Gordon Conference on Immunology and Immunochemistry, at the Belmont House Conference on the Development of Host Defenses and at the NIAID-BOB Symposium on Bacterial Vaccines. Dr. Ronald Schwartz chaired a workshop at the Midwinter conference of Immunologists at Asilomar, California. In addition, laboratory scientists presented seminars at major universities and research institutes in the United States and abroad.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00030-08 LI

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Antigen Recognition and Activation of Immunocompetent Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: William E. Paul, M.D., Chief, LI/NIAID

Other Investigators: Dr. Ethan Shevach, Senior Investigator, LI/NIAID
Dr. David Thomas, Guest Worker, LI/NIAID
Dr. Alan Rosenthal, Senior Investigator, LCI/NIAID
Dr. Irwin Scher, Clinical Immunology Division, NMRI
Dr. Aftab Ahmed, Clinical Immunology Division, NMRI
Ms. Susan Sharrow, Immunology Branch, NCI.

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The general goal of this project is to delineate the mechanisms involved in the regulation of the activation of thymus-dependent (T) and thymus-independent (B) lymphocytes. To this end, studies of the genetic regulation of T lymphocyte activation directed at an analysis of the role of molecules encoded in the major histocompatibility complex (MHC) have been carried out. These experiments strongly suggest that T lymphocytes possess antigen-binding receptors which recognize complex antigenic determinants involving both conventional antigen and an MHC structure expressed on an auxilliary cell. The other principal approach has been to analyze the requirements for B lymphocyte activation by studying the responses and surface markers of B lymphocytes derived from a strain of mice with an X-linked defect in B lymphocyte maturation or function.

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The studies performed under this project may be conveniently sub-divided into those dealing with the regulation of T lymphocyte activation and those concerning B lymphocyte differentiation and responsiveness. In studying the genetic regulation of T lymphocyte activation, we have principally concerned ourselves with the nature of the collaboration between antigen-pulsed adherent peritoneal exudate cells (PECs) and primed T lymphocytes. Rosenthal and Shevach had previously shown that activation of primed T lymphocytes, as measured by incorporation of ^{3}H-thymidine (^{3}H-TdR) into DNA, was efficient only if the antigen-pulsed PECs used to present antigen were syngeneic or semi-syngeneic to the T lymphocytes. Allogeneic combinations of antigen-pulsed PECs and primed T lymphocytes failed to yield proliferative responses. These studies were originally interpreted as suggesting the existence of cellular interaction (CI) structures which were encoded in the major histocompatibility complex (MHC). An alternative interpretation of this result is that T lymphocytes recognize a complex antigenic determinant (CAD) composed of the nominal antigen and an MHC gene product. Thus, in normal immunization procedures, T lymphocytes specific for CADs composed of antigen and syngeneic MHC gene products are primed; secondary challen e with antigen-pulsed syngeneic PECs presents T lymphocytes with the CAD to which they were originally primed while challenge with antigen-pulsed allogeneic PECs presents a non-cross reactive CAD.

If this concept were correct, it would be expected that (2x13)F_1 hybrid guinea pigs primed to ovalbumin or to purified protein derivative of tuberculin (PPD) should develop two non-cross reactive sets of primed T lymphocytes one responsive to CADs composed of nominal antigen and strain 2 MHC gene products and the other responsive to CADs based on strain 13 MHC products.

To examine this issue, we used both positive and negative selection techniques in which T lymphocytes from primed F_1 donors were cultured with antigen-pulsed strain 2 or 13 PECs. In the positive selection experiments cells were cultured for one week during which time non-stimulated cells died while stimulated cells proliferated. Upon restimulation, it was noted that F_1 cells from OVA-primed donors which had been initially selected with OVA-pulsed strain 2 PECs responded preferentially or exclusively to restimulation with OVA-pulsed 2 PECs, with little or no response to OVA-pulsed 13 PECs. Similarly OVA-primed F_1 cells initially cultured with OVA-pulsed 13 PECs thereafter responded preferentially to OVA-pulsed 13 PECs.

Similar results were obtained using a negative selection procedure in which proliferating cells were destroyed by treatment with bromodeoxyuridine (BUdR) and light. OVA-primed F_1 cells initially cultured with OVA-pulsed 2 PECs in the presence of BUdR and light were thereafter unresponsive to OVA-pulsed 2 PECs but responded normally to OVA-pulsed 13 PECs. These results indicate that the clone of T cells responsive to OVA-pulsed 2 PECs is distinct from the clone of cells responsive to OVA-pulsed 13 PECs and support that the CAD theory in contrast to the cellular interaction theory.

These experiments will be continued to more precisely analyze the 2 and 13 gene products which are critical to the distinction between antigen-pulsed 2 and 13 PECs. Attempts to isolate CADs from PEC membrane and to characterize T lymphocyte receptors will be extended.

These studies promise to be of substantial importance in the understanding of the process of antigen-recognition by T lymphocytes and in the function of specific immune response gene products. In turn, this should shed important light on immune responses to pathogenic agents and autoantigens in humans and to the understanding of associations between particular human MHC types and susceptibility to various diseases.

The second major group of studies included in this project deal with the ontogeny and differentiation of mouse B lymphocytes. The study of this problem has been markedly aided by the availability of a mutant mouse strain, the CBA/N strain, which has an X-linked defect in B lymphocyte function, presumably due to an arrest of B lymphocyte maturation. We have evaluated the density and class of immunoglobulin on the surface of B lymphocytes in the course of maturation and have noted certain very interesting findings. First, as reported last year, adult CBA/N spleen cells differ from normal adult spleen cells in the absence of a dominant population of lymphocytes with low to intermediate density of total surface immunoglobulin and in an abnormally low ratio of membrane δ chain homolog to membrane μ chain. This year, we have extended these studies to the characterization of the relative amounts of membrane μ chain on CBA/N and normal B lymphocytes and to a more detailed evaluation of B lymphocyte ontogeny in normal strains. Our results reveal that CBA/N cells have, on the average, a higher density of membrane μ chain than do cells of normal strains and that the density of μ on adult CBA/N spleen cells is precisely that of neonatal (3 day old) spleen cells of either normal or CBA/N mice. Furthermore, the development of splenic B lymphocytes in normal strains is characterized by a sequential transition from cells which have high average amounts of membrane μ, little or no δ and which lack both complement receptors and the antigenic determinants which stimulate Mls locus mixed lymphocyte responses into cells with less μ, substantial δ, easily detectable complement receptors and Mls determined antigen. In addition, immature B lymphocytes respond well to certain thymus (T)-independent antigens such as trinitrophenyl (TNP)-derivatives of Brucella abortus, but poorly or not at all to another set of T-independent antigens. More mature cells respond vigorously to certain T-dependent antigens to which immature cells respond poorly. Studies of the abnormality of CBA/N mice suggest that it lies in the failure of this adult population of B lymphocytes to develop and that the defect is intrinsic to the lymphocyte progenitors and not to the environment in which they develop.

Additional studies are planned to more precisely determine the cellular and molecular basis of the immune defect of CBA/N mice and to further evaluate the ontogeny of B lymphocytes in normal strains. These studies should provide important insight into the development of immune competence in humans and into the genetic regulation of cellular differentiation.

PUBLICATIONS:

Gelfand, M.C. and Paul, W.E. Prolongation of allograft survival in mice by administration of anti-Thy 1 serum. I. Mediation by in vivo activation of regulatory T cells. J. Immunol. 115: 1-4, 1975.

Ben-Sasson, S.Z., Paul, W.E., Shevach, E.M. and Green, I. In Vitro selection and extended culture of antigen-specific T lymphocytes. I. Description of selection culture procedure and initial characterization of selected cells. J. Exp. Med. 142: 90-105, 1975.

Gelfand, M.C. and Paul, W.E. The role of regulatory lymphocytes in prolongation of allograft survival by anti-Thy 1 serum. In Suppressor Cells in Immunity, (ed. by S.K. Singhal and N.R. St. C.Clair, Univ. of Western Ontario, London, Ont., 1975, p. 69).

Paul, W.E. Genetic control of specific interactions of immunocompetent cells. Speculation on mechanisms and general biological significance. Fed. Proc., in press.

Paul, W.E., Shevach, E.M., Schwartz, B., Schwartz, R., Finkelman, F.D. and Green, I. T lymphocyte receptors, antigen recognition and immune response genes. Proceedings of the 9th Miles Symposium, in press.

Scher, I., Steinberg, A.D., Berning, A.K., and Paul, W.E. X-linked B lymphocyte defect in CBA/N mice. II. Studies of the mechanisms underlying the immune defect. J. Exp. Med. 142: 637-650, 1975.

Ben-Sasson, S.Z., Green, I., Shevach, E.M., and Paul, W.E. Specific selection and functional enrichment of antigen-responsive T lymphocytes. In Membrane Receptors of Lymphocytes, (ed. M. Seligmann) 1975, pp. 311-321.

Finkelman, F.D., Smith, A.H., Scher, I. and Paul, W.E. Abnormal ratio of membrane immunoglobulin classes in mice with an X-linked B lymphocyte defect. J. Exp. Med. 142: 1316-1321, 1975.

Ben-Sasson, S.Z., Paul, W.E., Shevach, E.M. and Green, I. In vitro selection and extended culture of antigen-specific T lymphocytes. II. Mechanisms of selection. J. Immunol. 115: 1723-1730, 1975.

Ben-Sasson, S.Z. and Paul, W.E. Selection and enrichment of antigen-responsive T lymphocyte populations. A review. Israel J. Med. Sci., in press.

Elfenbein, G.J. and Gelfand, M.C. Proliferation of mouse bone marrow-derived lymphocytes in vitro: One mechanism of response to concanavalin A and phytohemagglutinin. Cell. Immunol. 17: 463-471, 1975.

Finkelman, F.D., van Boxel, J.A., Asofsky, R. and Paul, W.E. Cell membrane IgD. Demonstration of IgD on human cord blood lymphocytes by enzyme-catalyzed iodination and comparison with cell-surface Ig of mouse, guinea pig and rabbit. J. Immunol. 116: 1173-1181, 1976.

Janeway, C.A. Jr., Paul, W.E., Werblin, T.P. and Lieberman, R. Ig-G specific helper activity of T-lymphocytes from mice lacking the Ir-IgG gene. Immunogenetics, in press.

Scher, I., Sharrow, S.O., Wistar, R. Jr., Asofsky, R. and Paul, W.E. B lymphocyte heterogeneity: Ontogenetic development and organ distribution of B lymphocyte populations defined by their density of surface immunoglobulin. J. Exp. Med., in press.

Scher, I., Sharrow, S.O. and Paul, W.E. X-linked B lymphocyte defect in CBA/N mice. III. Abnormal development of B lymphocyte populations defined by their density of surface immunoglobulin. J. Exp. Med.. in press.

Scher, I., Ahmed, A., Sharrow, S.O. and Paul, W.E. Maturation of murine B lymphocytes in Development of Host Defenses, in press.

Paul, W.E., Shevach, E.M., Thomas, D.W., Pickeral, S.F. and Rosenthal, A.S. Genetic restriction in T lymphocyte activation by antigen-pulsed peritoneal exudate cells. Cold Spring Harbor Symposium Quant. Biol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00033-08 LI

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Lymphocyte Interactions, Receptors and Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Dr. Myron Waxdal, LI/NIAID
Dr. Dean Mann, Immunology Branch/NCI
Dr. Helen Hu, LI/NIAID
Dr. Theodore Konen, LI/NIAID

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INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.4	3.4	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

First, the relationship between eell surface histocompatibility (H) antigens and immunogenicity was investigated in a series of B cell leukemias of inbred strain 2 guinea pigs. It was found that the presence of certain H antigens, Ia antigens on the cell surface were necessary for the immunogenicity of the tumor associated transplantation antigens (TATA). A leukemic line that lacked this Ia antigen was not immunogenic despite the fact that it could be demonstrated to possess the TATA.

Second, Ia antigens are being detected on human leukemic cells. Human leukemic cells are being labelled internally with radioactive amino acids. The cells are then solubilized and the glycoproteins are absorbed to and eluted from lectin columns and then run on polyacrylamide gels. Molecules presumed to be Ia antigens can be detected by their characteristic mobility on the gels. In addition the cell extract can be precipitated by known alloantisera to human Ia antigens to determine whether the peaks observed by gel electrophoresis are actually Ia antigens.

This laboratory has been investigating a series of guinea pig leukemias of B cell origin (L2C leukemias). This leukemia first arose spontaneously 25 years ago in an old Strain 2 female guinea pig. Five subtypes of this leukemia have been identified on the basis of biological behavior, serological characteristics and karyotypic analysis.

Four of the 5 lines have a TATA as measured by the results of immunization protection tests. All 5 lines have an IgM surface antibody of identical idiotype. All 5 lines have a unique marker chromosome but each line has other karyotypic differences that suggest that each line is distinct. All these leukemic lines have on their surface the B alloantigen (equivalent of the murine H-2K and H-2D antigens) and four of these five lines have on their surface the Ia alloantigens normally present on the Strain 2 lymphocytes.

A correlation was then made among the lines for the presence of the Ia antigen and the presence of the TATA. The results indicated that the four Ia positive leukemia lines bear a TATA which is able to induce specific resistance to a subsequent challenge with the leukemia cells of the same line in Strain 2 hosts. In contrast, the Ia negative mutant line did not possess TATAs as measured by the same type of immunization and challenge experiments. However, criss-cross protection and challenge experiments demonstrated that it is not simply that Ia negative leukemia cells lack a TATA but rather the TATA of this particular mutant is not immunogenic. That is, Strain 2 animals preimmunized with cells of any of the Ia positive L2C leukemic lines can subsequently reject a challenge with the Ia negative leukemia cells. These results indicate that Ia negative cells share a common TATA with the other L2C leukemias, and that it can be recognized by immunized recipients, but that this TATA is not immunogenic when it is present on the Ia negative cell.

These findings are significant because they demonstrate that there is a close functional connection on the cell surface between Ia antigens and TATA. In the absence of Ia antigens the TATA of the Ia negative line appears to behave as a hapten. Strategies to successfully immunize against TATA and/or treat tumors by immunological methods may be improved by taking these observations into consideration.

Future directions: Attempts are being made to solubilize the surface membranes of the L2C cells to directly determine whether the TATA and the Ia antigens are physically associated. Also immunization experiments, in which cells rich in Ia antigens (strain 13 macrophages) are being mixed with Ia negative tumor cells, are being performed to determine whether simultaneous immunization with tumor Ia distinct cells and non-immunogenic tumor cells can induce specific immunity to L2C leukemia.

Finally since the L2C leukemias have a unique IgM on their surface and since we have an antibody to this particular idiotype, it is possible by immunofluorescent staining to detect a small number of L2C cells in the presence of a large number of normal lymphocytes. This technique is being used to detect the first appearance of the malignant L2C cells in a normal

animal injected with leukemic cells; if successful it will be a model for the early detection of leukemic cells in patients in early relapse of leukemia.

Next we have performed studies to defect and characterize Ia antigens on human malignant lymphocytes. If Ia antigens are found their presence and type will then be correlated with the type of leukemia and the clinical aspects of the disease. Furthermore the availability of human leukemic cells offer the possibility of obtaining large quantities of Ia antigens for their further detailed chemical characterization. Also such Ia antigens could be used to immunize animals so as to prepare large quantities of heteroantibodies to Ia antigens.

Ia antigens are cell surface antigens controlled by genes in the major histocompatibility (H) complex. Ia antigens are found in highest density on B lymphocytes and macrophages; however they can also be found on T lymphocytes as well as sperm and epidermal cells.

Ia antigens are closely associated with a number of important immunological phenomena including immune response gene (Ir gene) function, graft versus host disease, mixed lymphocyte reactions and transplantability of tissues and cells. Ia antigens consist of two chains with molecular weights of 25,000 and 33,000 Daltons, respectively. Recently hetero and allo antisera to human Ia antigens have become available.

Leukemic cells were collected from Hypaque-Ficoll gradients. They were then internally labelled with radioactive leucine and lysate were prepared by solubilization in detergent. The solubilized proteins were then passed over lentil lectin columns. The adherent glycoproteins were then eluted with specific sugars. The protein was then examined by polyacrylamide gel electrophoresis. The cells from three patients with the Sezary syndrome (a T cell leukemia), 7 patients with CLL (a B cell leukemia) and several normal individuals were examined for the presence or absence of Ia antigens.

Ia antigens could not be detected on normal peripheral blood mononuclear cells (probably because of the low % of B cells normally present). Similarly, cells from patients with T cell leukemias did not contain detectable Ia antigens. In contrast, the cells from all patients with CLL contained large amounts of Ia-like molecules; these had m.w. of 33,000 and 25,000 Daltons. In addition preliminary studies with Dr. D. Mann indicated that selected alloantisera against Ia antigens bound these molecules.

Future course of this project will involve further studies with these alloantisera to determine whether the Ia antigens of the cells from individual patients with CLL will react with only certain anti Ia sera. Such studies will greatly help in the identification of individual human Ia antigens. Eventually sufficient quantities of Ia antigens may be obtained so that amino acid sequence studies may be performed on these antigens. Further it is possible that important clinical correlation will be made between the presence or absence of Ia antigens on leukemic cells, or the presence of particular Ia antigens and the biological behavior of the leukemic cells.

PUBLICATIONS:

Forni, G., Rhim, R.S., Pickeral, S.F., Shevach, E.M., and Green, I.: Antigenicity of carcinogen and viral induced sarcomas in inbred and random bred guinea pigs. J. Immunol. 115: 204-207, 1975.

Gelfand, M.C., Frank, M.M. and Green, I. A receptor for the third component of complement in the human renal glomerulus. J. Exp. Med. 142: 1029-1036, 1975.

Ben-Sasson, S.Z., Paul, W.E., Shevach, E.M. and Green, I. In vitro selection and extended culture of antigen-specific T lymphocytes. II. Mechanisms of selection. J. Immunol. 115: 1723-1727, 1975.

Gelfand, J.A., Fauci, A.S., Green, I. and Frank, M.M. A simple method for the determination of complement receotor-bearing mononuclear cells. J. Immunol. 16: 595-602, 1976.

Rhim, J.S., Cho, H.Y., Kim, J.M. and Green, I. Induction and characterization of virus-free guinea pig tumors induced by Kirsten Sarcoma Virus. J. Nat. Cancer Inst. In press.

Berard, C.W., Gallo, R.C., Jaffe, E.S., Green, I. and DeVita, V.T. Current concepts of leukemia and lymphoma: Etiology, pathogenesis and therapy. Annals of Int. Med. In press.

Jaffe, E.S. and Green, I. Malignant lymphomas: Pathological, immunological and functional correlations in "Mechanisms of Tumor Immunity". Edited by I. Green, S. Cohen and R.T. McCluskey. J. Wiley & Sons, NYC. In press.

Frank, M.M., Jaffe, E.S. and Green, I. Detection of specific mononuclear cell receptors in tissue sections in "In vitro methods of cell mediated immunity", edited by John David and Barry Bloom. Academic Press, New York City. In press.

Forni, G. and Green, I. Heterologous sera: A target for in vitro cell mediated cytotoxicity. J. Immunol. In press.

Forni, G., Shevach, E.M. and Green, I. Mutant lines of guinea pig L_2C leukemia: I. Deletion of Ia alloantigens is associated with a loss in immunogenicity of tumor associated transplantation antigens. J. Exp. Med. In press.

Whang-Peng, J., Lee, E.C., Forni, G. and Green, I. Mutant lines of guinea pig L_2C leukemia: II. Comparative cytogenetic studies and banding analyses of normal and leukemickayrotypes. J. Nat. Cancer Inst. In press.

Gelfand, M.C., Shin, M.L., Nagle, R.B., Green, I. and Frank, M.M. The glomerular complement receptor in immunologically mediated renal glomerular injuries. N. Eng. J. of Med. In press.

Jaffe, E.S., Braylan, R.C., Frank, M.M. Green, I. and Berard, C.W. Heterogeneity of immunologic markers and surface morphology in "Childhood Lymphoblastic Lymphoma". Blood. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00034-04 LI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Differentiation and Activation of Mouse T and B Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any) LMI/NIAID, Department of Clinical and Experimental Immunology, Naval Medical Research Institute, Bethesda, Maryland 20014

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INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Lymphocytes in the mouse (as well as other species) are divided into two major functionally-distinct classes, T cells (thymus-derived) and B cells (bone marrow-derived). Our studies focus on: 1) differentiation of B cells in ontogeny and their relative sensitivity to triggering by antigens which are more or less thymus-dependent; 2) the defect in B cell maturation in the CBA/N mouse which appears to define a relatively immature B cell subclass; 3) the activation requirements for normal B cells, particularly their sensitivity to thymus-independent antigens, anti-immunoglobulin and anti-idiotype reagents; and 4) the influence of T cells at various stages of differentiation upon the responses of B cells.

Mice of the CBA/N strain have an X-chromosome linked abnormality in B-cell function manifested as an inability to respond to many T-independent antigens, among which are DNP-Ficoll and type III pneumococcal polysaccharide (S-III). Their B cells appear to have a surface immunoglobulin distribution similar to that of neonatal B cells of normal strain, namely an increased IgM to mouse IgD homologue ratio. CBA/N mice have low circulating IgM levels, and study of such mice may give some insight into human X-linked dysgammaglobulinemias as well as into B cell differentiation. We have found that CBA/N spleen cells can respond to some thymus-independent antigens in vitro or in vivo. These antigens are TNP-lipopolysaccharide (TNP-LPS) and TNP-Brucella abortus (TNP-BA). Sepharose or polyacrylamide beads conjugated with TNP will also stimulate CBA/N B cells in the absence of detectable numbers of T lymphocytes, i.e. in a T-independent fashion. The nature of these T-independent antibody responses in CBA/N mice was investigated and found to have three differences from the responses of congenic normal mice. Firstly, fewer B cells from CBA/N mice responded to the antigens; secondly, more antigen was required to elicit the peak response; and thirdly, the avidity of the anti-TNP antibody secreted by CBA/N B cells was lower then that of normal B cells. The properties of the B cells which CBA/N mice do possess have thus been further characterized and the classes of antigens to which they can and cannot respond better defined.

B lymphocytes of the neonatal mouse differ from B cells in the adult by a number of ways including surface immunoglobulin class and distribution, existence of C3 receptors and Ia antigens, and reactivity to antigen. The functional capabilities of neonatal B cells in terms of their ability to respond to different classes of antigens have been examined in detail in hope of elucidating the role of cell surface components in B cell activation. In addition, the susceptibility of neonatal splenic B cells to tolerance induction by the hapten-specific tolerogen, DNP-D-glutamic acid-D-lysine (DNP-D-GL) is being investigated. Neonatal B cells respond to TNP-Brucella or TNP-LPS before they can respond to TNP-Ficoll. Although all three antigens are thymic-independent in the adult mouse, activated T lymphocytes can substantially augment the plaque-forming cell response of neonatal B cells to these antigens. The ability of neonatal B cells, which lack or have very low numbers of cells bearing IgD or the C3 receptor, to be triggered seems to exist very early, but full amplification of B cell responses requires adult T cells or T cell factors.

The mechanism by which antigen triggers B lymphocytes to proliferate and secrete specific antibody is a central question in immunology. A number of approaches are being undertaken to address this question. The role of surface immunoglobulin (sIg) in B cell triggering has yet to be defined. Three major possibilities exist for sIg; 1) its interaction with antigen is required and is sufficient for B cell activation (1 signal model); 2) its binding to antigen is required but is not sufficient for triggering (2 or more signal hypotheses); and 3) it only serves to focus antigen and does not deliver any activating signal (focusing hypothesis).

Anti-immunoglobulin reagents are being employed to investigate the role of sIg in B cell activation. CBA/N B cells (described in Project

subtitle I) have been found to be much more susceptible than normal mouse strains to inhibition with a polyvalent goat anti-mouse MOPC-104E (μ,λ) reagent. The suppressive activity is largely directed at μ chain determinants since prior absorption of the antiserum with μ-bearing myelomas reverses the inhibition. The stimulatory activity of purified anti-Ig reagents coupled to inert beads is under investigation in both the mouse and the human with particular attention directed to the effects of anti-IgD (on its mouse homologue) antibody. If binding of sIg is sufficient to trigger B cells, then such anti-Ig reagents should be able to turn on antibody synthesis by large numbers of B cell clones.

A second approach involving anti-Ig reagents is the stripping and re-synthesis of sIg by normal and CBA/N B cells exposed to anti-polyvalent Ig reagent. Neonatal B cells have been found to re-express their sIg much more slowly than adult B cells following anti-Ig-induced stripping and internalization. CBA/N B cells did not behave as did neonatal cells with regard to the delayed re-expression of surface Ig despite the many functional similarities between CBA/N and neonatal B lymphocytes.

A third approach to understanding B cell activation has been to address the question of subpopulations of B cells responding to different antigens. For instance, are those B cells which respond to TNP on the T-independent carrier LPS the same as those which respond to TNP on Ficoll or TNP on KLH. The method chosen to address this question has been to stimulate mouse spleen cells in culture with one TNP-conjugate, eliminate responding cells by ^{3}H-thymidine suicide or BUdR and light treatment and re-stimulate the remaining cells with a battery of TNP-substituted antigens. Preliminary results suggest that both specific depletion and non-specific loss of suppression (perhaps T cells) can be induced by eliminating proliferating cells during the initial culture period. Addition of polyclonal activators like LPS also increase cell survival in al cultures. Despite these complexities, the technique of B cell suicide has been developed to a state where it should be possible to detect the extent to which B cell populations responding to the same hapten on different carriers overlap. Another approach to B cell heterogeneity is the question of whether T-independent antigens like TNP-Ficoll can stimulate B cell memory; in particular, whether TNP on one T-independent carrier can prime for an anti-TNP response elicited by TNP on a second T-independent antigen. TNP-aminoethylcarbamylmethyl-Ficoll priming three days prior to in vitro stimulation with TNP-Ficoll, TNP-LPS, or the T-dependent antigen DNP-KLH enhanced the response to all three antigens. This apparent B cell priming effect is short-lived (less than 10 days) and is lower in magnitude than priming induced by T-dependent antigens, but its effect is evident on both T-dependent and T-independent responses.

Antibody directed against combining site determinants uniquely identifies the product of a clone of B lymphocytes as well as the surface immunoglobulin on that clone. Such anti-idiotype antibody recently has been reported to mimic the action of antigen in terms of eliciting specific B as well as

T cell immunity. In addition, two groups have proposed that the regulation of an antibody response to a specific determinant may involve anti-idiotypic antibody feedback directed against the appropriate clones, the so-called "network" hypothesis. In order to address this question further, the response of BALB/c mice to phosphorylcholine (PC) determinants is being studied. PC elicits antibody in BALB/c mice which is predominantly of one clonotype which appears to be identical to the PC-binding IgA myeloma TEPC-15, hence the idiotype is abbreviated as T-15. We have purified T-15 myeloma protein and are preparing anti-T-15 antiserum against it. The anti-T-15 antibody has been shown to inhibit specifically the PC resonse of BALB/c mice. We are purifying T-15-positive cells via column chromatography in hope of showing that T-15 selected cells are enriched for anti-PC responses and that T-15 depleted spleen cells cannot respond to PC. If the network hypothesis is correct, the anti-T-15 response of T-15 depleted cells should be unregulated since the cell capable of making the appropriate anti-idiotype (T-15) has been removed. The appearance of altenative clonotypes in T-15 depleted cells also will be examined. Dr. Morse will be looking at the surface and cytoplasmic immunoglobulin of T-15 selected cells. The goal of this project is to identify the specificity and mechanism of feedback regulation (be it T or B cell mediated) of antibody formation.

The regulatory influence of T lymphocytes on B lymphocyte responses appears to undergo a change during ontogeny. Suppressor T cells seem to diminish in activity and helper T cells for T-dependent antibody response increase in the first few weeks of neonatal life in the mouse. Further detailed studies on subpopulations of neonatal T cells responsible for suppression are underway. These studies take advantage of the tropism of mouse thymic virus (MTV), a herpesvirus that causes extensive thymic necrosis when injected into newborn mice, for precursors of T cells involved in most functions but not suppression. The 5-10% of T cells surviving MTV infection are enriched for the ability to suppress the antibody response of adult B cells in vitro. T lymphocyte subpopulations are known to differ in their expression of the Ly series of mouse lymphocyte alloantigens. Normal neonatal T cells and those selected T cells surviving MYV infection are being characterized as to their Thy1 and Ly 1,2,3 expression using flourescein-conjugated specific anti-serum and the flourescein-activated cell sorter. These analytical techniques allow the description of the number of cells bearing such alloantigens and the relative amount of alloantigen per cell, and will enable us to assign neonatal suppressor T cells to either a new subclass or a previously defined subclass of T cells.

The second major approach to T cell function addresses the question of the nature of the T cell factor first described by Taussig and Munro. This factor which has antigen-binding capacity and expresses Ia but not Ig determinants, enables immature B cells to make IgM antibody to T-dependent antigens. Because the factor which is induced by exposing "educated" T cells to antigen in culture for a brief period of time, seems to work mainly on immature B cells and does not invoke IgG synthesis, we are proceeding with the hypo-

thesis that the factor may reverse tolerance induction in an immature B cell which is particularly sensitive to antigen-induced tolerogenesis. The suppressive activity of this factor on the IgG responses of mature B cells also is being examined, since the factor may be identical to that described by Tada et al. This line of inquiry should lead to a better understanding of T cell regulation of B cell responses and the surface components which are important in B cell activation or tolerization.

PUBLICATIONS:

Cohen, P.L., Scher, I. and Mosier, D.E.: In vitro studies of the genetically determined unresponsiveness to thymus-independent antigens in CBA/N mice. J. Immunol. 116: 301-304, 1976.

Mosier, D.E., Tigelaar, R.E. and Cohen, P.L.: Ontogeny of in vitro correlates of GVH reactivity. Transpl. Proc. In press.

Chused, T., Kassan, S. and Mosier, D.E.: Macrophage requirement for T-independent responses in vitro. J. Immunol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00035-03 LI

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Specificity in Immune Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Dr. Hillel S. Koren, I/NCI
Dr. John R. Wunderlich, I/NCI
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Dr. Ira Green, LI/NIAID
Dr. Alfred Steinberg, NIAMMD
Dr. Donald E. Mosier, LI/NIAID
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Dr. James Mond, LI/NIAID
Dr. Bruce Merchant, BOB/FDA
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COOPERATING UNITS (if any) Laboratory of Cell Biology, National Cancer Institute (Dr. Appella), Immunology Branch, National Cancer Institute (Drs. Koren, Wunderlich, Rehn and Shearer), Bureau of Biologies, FDA (Dr. Merchant).

LAB/BRANCH

Laboratory of Immunology

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INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords) The long range objectives of this project is to carefully re-examine the nature of binding specificity at antibody combining regions and other types of receptor sites. The effects of polyvalent binding on binding energy and specificity will be considered, and together these properties will be related to the behavior of immune systems. In particular, multispecific binding of disparate structures by individual antibody species is being studied through screening of specifically purified, radiolabeled antibodies with affinity chromatography columns. The ligands are bound to these columns using large haptenic reagents synthesized specially for this purpose using methods of peptide synthesis. Multispecific binding will be used for producing and isolating homogeneous antibody by cross-stimulation and immunoadsorption. The resulting preparations will be studied by amino acid sequencing in order to gain more insight into the nature of antibody diversity. Methods for improving sequence analysis are actively being pursued.

A number of chemical techniques and products that have been developed from the above work are being used in collaborative studies on molecular interpretations of cellular mechanisms in immune responses. Materials employed include synthetic thymus-independent antigens.

The overall objective of the project is a more complete description of the molecular basis for specificity in immune responses. The binding properties of antibodies and other receptor-bearing molecules are to be studied and related with the observed behavior of immune systems.

Objectives of work carried out under this subtitle are (1) to explore the nature and frequency of binding of unrelated antigenic structures by individual antibody molecules, (2) to employ multispecific binding as a means for producing restricted antibody responses and obtaining immunoglobulins suitable for structural studies, (3) to use multispecific binding and amino acid sequence analysis of immunoglobulin variable regions in studies of the genetic basis for diversity.

The frequency and generality of multispecific binding by antibodies will be explored by use of small affinity chromatography columns to which are bound a variety of large haptenic structures. Specifically purified antibodies will be labeled with ^{125}I-active ester and passed through these columns. Any antibody species that are bound will be detected by counting radioactivity on the columns and from effluents after elution with corresponding soluble haptens. With suitable modification of technique affinity columns can be used for estimating binding constants of bound proteins. Multispecific antibodies will be examined by isoelectric focusing in order to assess structural restriction.

During the past year work has been directed toward the systematic synthesis of large haptenic reagents. These reagents are designed to be large enough so that they may fully occupy most antibody combining sites; they will possess various structural features and a functional group for attachment to adsorbents or other carriers. In recent months our main effort has been to perfect the synthesis of glutamic acid derivatives which allow the assembly of various branched haptens with hydrophilic spacer arms. The haptens now being prepared are comprised of 4 to 6 amino acid residues and will eventually carry a variety of terminal groups.

Studies in this laboratory and elsewhere have shown that some antibody molecules do possess several distinctly different specificities. The question remains unanswered whether (1) an occasional antibody may have several subsites that can bind distinctly different structures, or else the several structures have inapparent similarities, (2) most antibody molecules can bind a few disparate antigens, or (3) most antibodies can bind a great many distinctly different structures but with a frequency lower than experiments heretofore have revealed. The latter case, that of general multispecificity, is an attractive hypothesis from theoretical considerations. I have recently developed some models of binding frequency relationships including th e equilibrium binding constant parameter which are consistent with known facts and the principle of general multispecificity. The above fundamental question about antibody specificity, which should apply to other kinds of receptor molecules, will be pursued by the experimental methods outlined above. The type of multispecificity exhibited by antibodies has an important bearing on the mechanisms that general structural diversity regarding the extent of

diversity required.

Considerable effort has been devoted to improving methodology in amino acid sequencing. The method for producing new identification derivatives from the Edman degradation was developed further. This method, which originated in my laboratory, yields the N-phenylthiocarbamyl (PTC) alkylamides of amino acids by the rapid reaction of an aklylamine with thiazolinone resulting from sequential degradations. Some important advantages are gained over the use of conventional PTH-amino acid derivatives, chiefly as the improved recovery of certain commonly occurring amino acids. Intensive study of the PTC methylamides (PTMA amino acids) has been carried out. Principal findings are (1) excellent conditions for separating these derivatives on thin layer plates along with a high sensitivity method for their detection (0.05-0.5 nmoles), (2) high resolution separations on thin layers in the programmed multiple development apparatus, (3) recovery of serine and threonine PTMA's in 57 and 75% recoveries respectively, (with unaltered side chains), from solid-phase Edman degradations, (4) demonstration of rapid, effective conversion of dry thiazolinones to PTMA's by exposure to methylamine vapor, and (5) important knowledge of stability conditions of thiazolinones prior to conversion to PTMA amino acids. Standard solutions of the latter have been conveniently prepared from PTC-amino acid resin amides. Synthesis of the latter was developed during the past year. Solutions of the standards proved to be stable in the refrigerator for many months. An automatic conversion device has been designed and installed on the automated solid-phase sequencer (Sequemat Corp.). The testing of this device is currently underway in Dr. Appella's laboratory. The design was a joint effort between Dr. Appella and myself, the Sequemat Corp. and the NIH instrument shop.

Some work was initiated on improved approaches to solid-phase sequencing. Promising new supports were designed and synthesized which are based on a non-swelling porous glass matrix. It is hoped that the new features will overcome disadvantages so far encountered in other laboratories that have used porous glass supports. A micro-column technique for manual solid-phase sequencing was developed and used successfully in sequencing small peptides. The technique was also useful in testing new supports and in exploring some novel, non-aqueous, PTC coupling systems. A demand for improved methodology in amino acid sequencing is increasing in a number of areas of biochemistry. The need for greatly increased sensitivity is especially acute in the field of membrane protein biochemistry and genetics where one is limited to very small samples. Greater sensitivity and speed are required for the aims of this project, i.e., to explore the nature and extent of antibody diversity. Work will continue on development of solid-phase techniques of sequencing in conjunction with the use of PTMA derivatives. Principal objectives for the coming year are (1) development of a high-pressure liquid chromatographic separation of PTMA amino acids, (2) trial sequencing of peptides by an automated solid-phase method with automatic conversion, (3) development of a high through-put manual solid-phase sequencing method, (4) adaptation of the PTMA method of identification to automated liquid-phase sequencing. Ideas for accomplishing these goals are at hand, and work is in progress.

Synthesis and/or Chemical Modification of Antigens: Studies of Specific Immune Responses at the Cellular Level.

Studies have been completed on the specificity of the in vitro cytotoxic response to hapten-modified, syngeneic and allogeneic murine lymphocytes. The work was carried out principally in the Immunology Branch of NCI by Drs. H.S. Koren, J.R. Wunderlich, T.G. Rehn and G.M. Shearer with whom I was a collaborator (see publications). For these studies two large haptenic reagents prepared in my laboratory, were employed for modifying stimulator and target cells. Specific stimulation and testing for cytotoxic effector cells were generally carried out in culture. Cytolysis was measured by the release of bound ^{51}Cr. The large haptenic groups employed were: N-(3-nitro-4-hydroxy-5-iodophenylacetyl)-β-alanylglycylglycyl (N) and p-arsonophenylazo-N-acetyl-L-tyrosylglycylglycyl (A) in addition to the trinitrophenyl (TNP) hapten.

In brief, these studies showed that for maximum cytolysis to occur the stimulating and target cells had to be matched with respect to both the hapten and one or both ends (K and/or D) of the major histocompatibility complex. The data were consistent with the hypothesis that the antigen recognized by the effector cells includes an altered H-2 serological cell surface product. Only very little cross-reaction was observed between N and A or between N and TNP. In vivo stimulation with N-modified, syngeneic spleen cells resulted in the formation of memory cells for the cytotoxic response in vitro. Memory, which lasted at least two months, was shown to be T cell dependent and hapten-specific both for target cells in the lytic reaction and for stimulator cells in the secondary response. Memory cells were not in themselves cytotoxic. All tests indicated that cytotoxic cells are T cells.

In another collaborative study carried out with Dr. Bruce Merchant, it was found that antibody-mediated suppression of a hapten-specific immune response occurred in both normal and athymic (nude) mice. However, important differences were noted. In the latter susceptability to suppression occurred over a very restricted time interval. In normal mice near-optimal suppression was obtained by administering specific antibody (in these studies, to hapten A) up to 48 hours after immunization, whereas in athymic mice suppression was only partially effected when antibody was given one hour after immunization. Presumably, T cells, when present, may serve to detain most antigen up to 48 hours while allowing suppressive antibody to interact with the antigen. Once free antigen has reached the B cell surface it may be largely refractory to the effect of suppressive antibody. Little has previously been known about T cell participation in antibody-mediated suppression. This type of suppression has been successfully exploited in the control of erythroblastosis fetalis. Further work will be carried out on antibody-mediated suppression in normal mice with both thymic dependent and independent antigens in order to clarify better the role of T cells.

Large haptenic reagents have been used effectively in studies on the mechanisms of B cell activation by Dr. Merchant in another collaborative project. An analysis was made of plaque-forming cell responses of CBA/N mice and suitable hybrids and backcrosses to in vivo immunization with a thymic independent antigen, A_{45}-Ficoll. Results clearly established that the genetic defect in the immune response to CBA/N mice to large-hapten Ficoll conjugates is X-

linked as has been shown for other thymic independent antigens by other workers. In addition, it has been shown that, although CBA/N mice can respond well to any hapten coupled to a thymic dependent carrier, simultaneous immunization with the same hapten coupled to Ficoll and given in minute amounts resulted in a total blockade of the otherwise normal immune response. This blockade was shown to be hapten specific, and it appeared not to be attributable to suppressor T cells. The blockade was not observed in normal mice nor did it extend to memory cells in CBA/N mice. The latter could mount a normal secondary immune response to a thymic dependent antigen - including "switchover" from IgM to IgG secreting cells.

Large haptenic probes and Ficoll conjugates (see previous annual report) continue to play important roles in a variety of immunological studies. A tripeptidyl derivative of phosphorylcholine bound to various proteins and polymers (Con A, Ficoll, D-GL and polyacrylamide particles) is being studied by several postdoctoral fellows and by Dr. Mosier in the Laboratory of Immunology.

A conjugate of Ficoll and deoxyribonucleotides was recently prepared in my laboratory and radioiodinated by the Bolton-Hunter procedure. Both the conjugation and labeling were carried out in a non-aqueous medium (dimethylformamide) with improved efficiency. Ficoll conjugates are soluble in 50% saturated ammonium sulfate and can be used in a Farr-type assay. Recently, the nucleotide conjugate has been used successfully to measure antinuclear antibodies raised in mice. These experiments were done in collaboration with Drs. Ira Green and Alfred Steinberg (NIAMDD). Radiolabeled Ficoll conjugates may find wide applicability in Farr-type radioimmunoassays.

In conclusion, it appears that tailored synthesis of antigenic reagents will continue to provide useful and often powerful tools for investigating mechanisms of immune responses. Eventually such special materials may play decisive roles in the rational control or manipulation of immune responses involved in many diseases. Plans are being made to employ a number of haptenic reagents that are now being prepared for specificity studies in future collaborative efforts such as those described above.

PUBLICATIONS:

Koren, H.S., Wunderlich, J.R. and Inman, J.K. In vitro development of cytotoxic T cells specific for hapten-associated antigens on syngeneic cells. Transplantation Proceedings 1 (No. 1, Suppl. 1): 169, 1975.

Inman, J.K. and Appella, E. Identification of thiazolinones from solid-phase Edman degradations after their conversion to PTC-amino acid alkylamides. In Solid-Phase Methods in Protein Sequence Analysis - Proceedings of the First International Conference (Richard A. Laursen, editor). Pierce Chemical Co. (Publisher) p. 241, 1975.

Rehn, T.G., Shearer, G.M., Koren, H.S. and Inman, J.K. Cell-mediated lympholosis of N-(3-nitro-4-hydroxy-5-dodophenylacetyl)-β-alanylglycylglycyl-modified autologous lymphocytes. Effector cell specificity to modified

cell surface components controlled by the H-2K and H-2D serological regions of the murine major histocompatibility complex. J. Exp. Med. 143: 127, 1976.

Koren, H.S., Wunderlich, J.R. and Inman, J.K. T cell memory for the cytotoxic response to hapten-modified target cells. J. Immunol. 116: 403 1976.

Merchant, B., Inman, J.K. and Lizzio, E.F. Participation of thymus-derived cells in the maintenance of antibody-mediated selective immune suppression. In The Ortho Symposium on Rh Immune Globulin. Ortho Pharmaceutical Corp. In press. 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00036-11 LI

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunoglobulin Genetics: Regulation of Gene Expression and Lymphoid Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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M. and S. Carta, Istituto Superiore Sanita, Rome Italy

COOPERATING UNITS (if any) In addition a large number of investigators at NIH and elsewhere received rabbits reagents and allotyping information from our laboratory.

LAB/BRANCH
Laboratory of Immunology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to understand the mechanisms which lead to the synthesis of highly specific antibody molecules, the nature of genes which influence and control immune responses, and the regulation of gene expression during lymphoid cell differentiation. We are attempting to define by combined immunological and chemical approaches the structural differences between genetically controlled polymorphic forms of rabbit immunoglobulins (immunoglobulin allotypes) and between classes, subclasses, types and subtypes of their polypeptide chains. We utilize such information in a variety of studies including breeding experiments, studies of the factors which influence phenotypic expression of alleles or chain types, and studies of membrane-associated markers.

Introduction

We are attempting to define by combined immunological and chemical approaches the structural differences between genetically controlled polymorphic forms of rabbit immunoglobulins (allotypes) and between classes subclasses, types and subtypes of their polypeptide chains. For these studies we prepare normal immunoglobulins and their polypeptide chains as well as antibodies specific for various bacterial and synthetic antigens as a source of allotype-bearing antigens. We prepare large amounts of antibodies specific for the various allotypes. The structural information and the reagents we prepare are utilized in planning and conducting a variety of experiments such as breeding to investigate linkage relationships, investigation of factors which influence phenotypic expression of alleles and chain types, studies of membrane associated markers and of immune responses. The overall objective is to understand the genetic mechanisms which lead to the synthesis of highly specific antibody molecules, the nature of the genes which influence and control immune responses, and the regulation of gene expression during lymphoid differentiation.

Although the a and b allotypes behave as if controlled by simple Mendelian alleles at the independent a and b loci, the polypeptide chain structures which lead to their serological distinction have been evolving for a long time. Most anti-b4, -b5 and -b6 antisera cross react with determinants which reside on the same hare light chains. Hare immunoglobulins, when injected into b4, b5, or b9 rabbits, elicited formation of antibodies which reacted with hare IgG and cross reacted with those rabbit allotypes (except b9) absent from the recipient. Cottontail rabbits exhibit polymorphisms of homologues of rabbit b5 and b9. Complex evolutionary pathways involving gene duplications, sequence divergence, intragenic recombinations and polymorphic regulatory genes have been proposed to explain the observed complexities. (Landucci Tosi, S., Mage, R. and Tosi, R.M., J. Immunol., in press, 1976a, 1976b).

The a allotypes represent groups of antigenic determinants expressed on different subpopulations of V-regions. Hyperimmunization can alter the relative expression of such populations and large amounts of Igs with unusual V-regions may be produced. Unusual IgG from an a1a3 rabbit hyperimmunized with M. lysodeikticus did not precipitate with a1a3 or a1 anti-a2 but precipitated strongly with some a3 anti-a2. Such anti-a2 antibodies also react strongly with sera of hares and cottontails. (Mage et al. presented at Homogeneous Immunoglobulins Workshop March 1 and 2, 1976).

Immunological properties of peptides from heavy chains of a3 allotype were studied using a radioimmunoassay that involved inhibition by these peptides of a reaction between ^{125}I labeled anti-a3 antibody and Sepharose bound a3 IgG. Heavy chain (H) lost some activity upon isolation from IgG and further activity was lost upon citraconylation of the lysine side chains (CH). The molar concentration of H and CH required for 50% inhibition of

(CH). The molar concentration of H and CH required for 50% inhibition of the a3-anti-a3 reaction were 5 and 55 times the concentration of H as it occurs in whole IgG. The corresponding differences in ΔF^{o} values were 0.96 Kcal/mole and 2.39 Kcal/mole. Digestion of the CH with trypsin caused a loss of activity corresponding to a $\Delta(\Delta F^{o})$ value of 0.57 Kcal/mole. Purification of the peptides from the whole tryptic digest followed by a second tryptic digestion resulted in a gain of activity equivalent to 1.3 Kcal/mole because of the deblocking of lysine side chains. Immunoabsorbent purification of immunopeptide T2 resulted in a loss of activity equivalent to 1.89 Kcal/mole. It is probable that a major contribution to the change in ΔF^{o} comes from losses in conformation at each step. Primary structures which contributes to some a3 antigenic determinants may also have been removed. (A.A. Ansari, M. Carta-Sorcini, E. Appella and R.G. Mage, Submitted).

Immunopeptide T2 with major a3 allotypic determinants was found to have a molecular weight of approximately 6000 which decreased to about 3000 after reduction and alkylation. NH_2- and COOH- terminal analyses and cysteine peptide mapping demonstrated that T2 is composed of two poly-peptide chains linked by a disulfide bond, one from the cys 22 region having lys at the C-terminus and one from cys 92 region ending with C-terminal arg 94. V_H residues 17-33, 64-79, and 84-85 of the isolated peptide are exposed on the surface of a hypothetical three-dimensional model of the Fv of a rabbit antibody molecule. The allotypic determinants may be far removed from the antibody combining site. (Carta-Sorcini, M., Ansari, A.A., Mage, R.G. and Appella, E., submitted)

Using a technique for rapid isolation of tryptophan-containing peptides after modification of the tryptophanyl residues, peptides from normal light chains of allotypes b4, b5 and b6 containing tryptophan 146 in the kappa chain constant region were isolated and their sequences compared with published sequences for b4 and b9 constant regions. Between b4 and b5, there are 7 allotypic variations in a total of 24 residues. A higher degree of homology exists between b5 and b6 where only one difference in ten residues is found. Another difference probably occurs between b5 and b6 at position 148. Since the b6 chain was split by trypsin at lysine 147, the proline in b5 at 148 is probably replaced by another amino acid in b6. The published b9 sequence appears to contain an inserted residue after proline 140 compared to the other b allotypes and differs from b4 and b5 by 11 or 12 residues at 14 or 15 positions in a stretch of 25 residues. The sequence differences found in the portion of the constant region which includes Trp 146 are consonant with results of our serological studies (Landucci Tosi et al., 1975) which predicted that b9 light chains would differ most in sequence from light chains of the other types and that b5 and b6 would be most similar in sequence. (A. Chersi, E. Appella, and R. Mage, in preparation).

Further refinement of a radioimmunoassay for b allotypes and anti-allotype antibodies has allowed detection of antibody concentrations as low as 5 ng/ml and also allows detection of anti-idiotype antibodies. Analyses of the responses of neonatal rabbits to maternal b-allotypic antigens reveals a short lived period of "tolerance" extending to only the second week

of life. Following this time, relatively normal immune responses to b antigens are observed. Immunization during the "tolerant" period may induce a prolonged period of unresponsiveness (Werblin, Young-Cooper and Mage).

Isoelectric focusing of purified b9- anti-b5-allotype antibodies produced by a single animal during an 18 month period after a single immunization and a boost at 1 year of age, reveals a stable pattern of relatively restricted heterogeneity. We are now investigating whether such restricted heterogeneity is a more general phenomenon in this type of response (Werblin and Mage).

Immunogenetics is providing new insights into the nature of structural and regulatory genes in complex mammalian systems. The chemistry, genetics, and evolution of immunoglobulins appears to be analogous in many respects to the chemistry, genetics and evolution of cell surface isoantigens such as those involved in transplantation, cell-cell recognition systems, and possibly some tumor antigens. Thus in addition to defining the genetic mechanisms which lead to the synthesis of highly specific antibody molecules, the chemical and genetic definition of immunoglobulin allotypes provides models which may have direct relevance to evolution and immunogenetics of cell surface isoantigens. Understanding of the chemistry, genetics and evolution of immunoglobulins, the factors which control their genetic expression, and their biological roles is thus of broad fundamental interest.

Most of the studies described are continuing. In addition we are attempting to define the structure and genetics of rabbit β_2-mico globulin and investigating mechanisms of allotype suppression in rabbits. In order to improve our record-keeping and better utilize the body of genetic data contained in the pedigrees of our rabbit colonies, we are collaborating with Dr. D. Alling, NIAID on computerizing our records. We hope to develop programs which will perform various genetic analyses of the data.
Appendix - Contract Number NO1-AI-81406 Maintenance and Breeding of Rabbits of Known Genotype and their Use for Immunological Studies

Funding: Man Years Purchased: 4

The objectives and methods are as outlined above. The contract facility performed over 1500 injections, 5500 bleedings as well as other miscellaneous technical procedures. Various short-term projects and 30 long-term experiments involving breeding, immunization and suppression were conducted. The contract facility housed and fed an average daily inventory of 500 rabbits. They carried out a weekly breeding program and weaned and tattooed approximately 500 young rabbits. They maintained records on all the above mentioned activities. Rabbits and reagents produced and supplied through this contract were made available to numerous other investigators at the NIH and at laboratories throughout the world.

PUBLICATIONS:

Landucci, Tosi, S., Tosi, R.M., Mage, R. and Young-Cooper, G.O.: Serological Relationships between rabbit immunoglobulin allotypes controlled by the b locus. Immunochem. 12: 865-872, 1975.

Mage, R.G.: Allotype suppression in rabbits: Effects of anti-allotype antisera upon expression of immunoglobulin genes. Transplantation Reviews 27: 84-99, 1975.

Landucci-Tosi, S., Mage, R. and Tosi, R.M.: The homologues of rabbit b allotypes on hare immunoglobulin light chains: An evolutionary puzzle. J. Immunol. In press.

Landucci-Tosi, S., Mage, R. and Tosi, R.M.: A homologue of the rabbit kappa chain allotype b9 on IgG from a cottontail rabbit. J. Immunol. In press.

Ansari, A.A. and Mage, R.G.: An evaluation of effectiveness of Bio-Glas in molecular sieving of polypeptides in guanidine hydrochloride. Analytical Biochem: In press.

Mage, R.G., Young-Cooper, G.O., Rejnek, J., Ansari, A., Alexander, C., Appella, E., Carta-Sorcini, M., Landucci-Tosi, S. and Tosi, R.: Rabbit immunoglobulin allotypes: Complexities of their genetics, expression, structural basis and evolution. Cold Spring Harbor Symp. Quant. Biol. 41: (invited paper).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00037-09 LI

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less

Immunogenetics of Mouse Immunoglobulins and Genetic Control of Antibody Response.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Homologous antisera prepared with homogeneous immunoglobulins (myeloma proteins) or normal immunoglobulins identify immunoglobulin IgCH allotypes and Fab idiotypes in the mouse. These antisera have been used in a variety of genetic studies involving tests for linkage, dominance and recombination. Specific anti-idiotype antisera have been particularly useful in studies of variable region site associated cross specific idiotypes on myeloma proteins that bind the same haptens. NOrmal antibodies made to the same hapten in some strains share these idiotypes; in others they are absent. The genes controlling these idiotypes were found to be linked to the Ig-CH region. By determining the frequency of recombination between IgV_H ang IgCH genes and between different VH genes, it may be possible to estimate the number of genes required for the diversity of antibody. In addition to studies of the structural genes for immunoglobulins it is also important to understand the mechanisms that control the response to particular antigens. The homogeneous immunoglobulins (myeloma proteins) are useful as immunogens to study the genetics of the immune response. The response to particular classes of immunoglobulins e.g. IgA and IgGzb have been found to be controlled by the H-2 linked immune response genes Ir-1a and Ir-1b. With the availability of NZB myeloma proteins having IgC_H genes

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different from those of BALB/c, it is now possible to determine if the same or different Ir genes are used to respond to IgA and IgGzb proteins derived from different genomes. With the continuing development of new H_2 congenic and recombinant strains it is possible to more closely define the I regions which carry the particular Ir genes.

Genetics of V-regions

Antibodies to specific haptens raised in mice often carry cross-specific antigenic (idiotypic) determinants. This is particularly relevant to immune responses in which the same subclasses of VL and VH chains appear evoked. Many of these cross-specific antigenic determinants appear to be polymorphic inasmuch as the antibodies of some strains regularly carry the idiotypes while in others they are completely absent. These phenotypic differences can be used as genetic markers in linkage studies and also in the mapping of the V_H genes.

We recently characterized nine myeloma proteins that bound specific haptens, e.g., polyfructosus of β2→1 linkages (inulin) and β2→6 linkages (bacterial levan). We described a complex system of cross-specific idiotypes that were shared by these myeloma proteins, many of which were site related and inhibitable by the hapten. We found that the cross-specific idiotypes of inulin-binding myeloma proteins were also present on natural antibodies to inulin and further that some strains carried these anti-inulin idiotypes while others did not.

Different strains of mice were immunized with bacterial levan and their anti-inulin (β2,1 fructosan) antibodies were examined for specific idiotypic specificities (IdX's) that are found on BALB/c myeloma proteins that bind inulin and bacterial levan. We identified two strains, BALB/c whose β2,1 fructosan antibodies carried the IdX specificities and C57BL/6 whose antibodies did not. This enabled us to study the genes designated Inu IdX controlling the inulin (β2,1) IdX determinants, for linkage to known immunoglobulin genes. An F_1 cross of BALB/c and C57BL showed the presence of Inu IdX in all the progeny indicating the codominance of the marker. The availability of the recombinant inbred (RI) and Ig congenic strains related to BALB/c and C57BL permitted linkage studies of the Inu IdX genes to the IgC_H allotype locus. The findings in these strains indicated that Inu IdX is a V_H marker linked to the BALB/c IgC_H locus. The strain distribution of the marker was determined. Progeny tests were also done to map the Inu IdX markers by determining the frequency of recombination. BALB/c has the a1 allotype and is positive for the Inu IdX marker. C57BL/6 has the a^2 and is negative for Inu IdX. 168 C57BL/6 x (C57BL/6 x BALB/c)F_1 mice were typed for the Inu IdX markers and for allotypes. Two mice carrying the a^2 allotype and the Inu IdX markers were found indicating a crossover frequency of 2/168 or 1.3%. These two recombinants were mated to DBA/2 mice which are negative for the Inu IdX marker and which carry the a^3 allotype. 6/15 of the resulting progeny also carried the Inu IdX marker indicating that recombination had indeed occurred.

Although genetic studies of the RI and Ig congenic strains for the Inu IdX markers indicated that these were V_H markers linked to allotype, the role of the light chain in the expression of the idiotypic determinants was important to ascertain. Recombinant molecules containing the light chain of one inulin binding myeloma protein and the heavy chain of another inulin binding myeloma protein are being examined to determine if the IdX's require both light and heavy chains, or heavy chains alone or light chains alone to be expressed.

These studies involved the preparation of an homologous system of anti-idiotype antisera specific for each myeloma protein. Antibody assays by hemagglutination and hemagglutination inhibition methods; ioslation and purification of anti-inulin and anti-levan antibodies on affinity columns; allotype typing; mating of specific progeny; preparation of different oligosaccharide haptens; and isolation of myeloma proteins on DEAE columns.

Studies of IgCH and IgVH Markers in NZB Mice Using Homogeneous Immunoglobulins (Myeloma Proteins)

NZB have a different IgG nome than BALB/c, NZB myeloma proteins are now available and have been found to carry some allotypic determinants that are different from BALB/c. Homologous antisera were prepared to the allotypes and idiotypes present on four different classes of myeloma proteins from NZB mice. New allotypic determinants have been found. The genetics of these new IgCH markers are being studied as well as the strain distribution. NZB myeloma proteins have been found that bind the same haptens as BALB/c myeloma proteins. The idiotypes found in normal antibody to these haptens and their relationship to BALB/c and NZB myeloma protein idiotypes that bind the same haptens are being investigated. In addition, in NZB the predominant myeloma protein obtained is of the IgG_{2a} type and some of these bind the same hapten. So far no shared idiotypes have been found among IgG_{2a} myeloma proteins of BALB/c that bind the same hapten. The idiotype of NZB myeloma proteins and their relationship to normal antibodies produced to the same hapten are now being investigated.

The following techniques have been used in these studies: preparation of anti-allotype and anti-idiotype antisera in an homologous system which involves selection of the appropriate strains for both the Ir and IgCH genes; isolation of myeloma proteins on DEAE columns; identification of allotypes and idiotypes by agar gel diffusion and hemagglutination and hemagglutination inhibition; immunoelectrophoresis; appropriate genetic studies to determine linkage and dominance, etc.

Genetic Control of Antibody Response to IgG_{2a} Myeloma Protein Having Kappa and Lambda Light Chains.

The immune response (Ir) to BALB/c IgG_{2a} myeloma proteins in the mouse is controlled by an Ir gene (Ir1b) linked to $H2^a$. Recently there has been some question as to whether the Ir 1b is in a region separate from the Ir 1a and

whether it maps in the same region as Ir-1a. New recombinant H_2 strains are now available which will permit us to re-examine this question. We will attempt to map the Ir 1b gene to a more defined region in the H_2. There is also intriguing evidence suggesting that multigene systems may control immune responses to IgG_{2a} kappa and IgG_{2a} lambda myeloma protein; this has prompted us to retest new recombinants that are now available as well as to do studies of the immune response in appropriate progeny.

Before Regulation of the Expression of IgCH Allotypes of the Mouse

These studies involve preparation and isolation of the myeloma proteins LPC1 and HOPC1 on DEAE columns, immunization of a large number of inbred congenic and recombinant strains of mice, antibody assay for allotypes and idiotypes by agar gel, hemagglutination and hemagglutination inhibition techniques, and progeny tests for genetic studies, etc.

Regulation of the Expression of IgCH Allotypes in the Mouse.

It has been noted for some time that in the production of Ig congenic strains there is sometimes decreased levels of particular classes of immunoglobulins. For example, the concentrations of BALB/c allotypic determinants on mouse Ig1 and $Ig2_a$ immunoglobulins in (BALB/c x CB20)F_1 mice are markedly lower than in BALB/c mice. Apparently the selection of a foreign genetic environment (example BALB/c) to express the allotypes of C57BL has a marked effect on the expression of allotypes. This phenomenon has been observed in allotype systems involving IgCH genomes other than C57BL and BALB/c, e.g. CAL20. Experimental models involving crosses of C57BLxBALB/c F_1 backcrossed to either BALB/c or C57BL and selecting for the C57BL and BALB/c allotypes respectively for many successive generations are being studied for their effect on the quantitative expression of the allotypic determinants on four different classes of immunoglobulins including Ig1, IgG_{2a}, IgG_{2b} and IgA. It is within the scope of these experiments that genes regulating the expression of structural IgCH gene may be identified. Such studies involve quantitation of allotypes and idiotypic determinants using radioimmunoassays; long term mating and progeny testing, characterization of antibody produced by isoelectric focusing, etc.

PUBLICATIONS:

Lieberman, R., Potter, M., Humphrey, W., Jr., Mushinski, E.B. and Vrana, M.: Multiple individual and cross-specific idiotypes on 13 levan-binding myeloma proteins of BALB/c mice. J. Exp. Med., 142:106-119, 1975.

Riblet, R., Blomberg, B., Weigert, M., Lieberman, R., Taylor, B.A. and Potter, M.: Genetics of mouse antibodies. I. Linkage of the dextran response locus V_H-Dex to allotype. Eur. J. Immunol. 5:175, 1975.

Lieberman, R., Potter, M. and Humphrey, W., Jr.: Genetics of β2→1 fructosan (Inulin) antibodies in mice. Immunogenetics. In press.

Lieberman, R. and Potter, M.: Presence of T15-S63 idiotype on M511. Immunogenetics. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00038 04 LI

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Structural and Activity Studies on Immunologically Important Cells and Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.8	3.0	1.8

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is to delineate the mechanism of mitogen stimulation of B and T lymphocytes by an analysis of the mitogen-membrane interactions and the mechanisms by which these interactions initiate or suppress cellular response. To this end we have isolated and identified the major cell surface acceptors for both mitogenic and non-mitogenic lectin on both T and B cells from mice. Further studies by blocking and/or capping these acceptors with antibodies, and by chemical modification of mitogens suggest that there is not a critical receptor, but that cell stimulation is triggered by a more general and complex set of interactions. These studies have been carried out with murine lymphocytes and now are being expanded to include human cells. Preliminary results indicate that human lymphocytes respond somewhat differently than their murine counterparts. The carbohydrate specificity of Pa-2, the major mitogen from pokeweed, has also been determined.

In collaboration with other units of NIH, work is in progress in the chemical structure of secretory IgA, especially the binding of secretory component and on the structure and origin of amyloid proteins of unknown origin. Studies on the primary sequence of guinea pig B, S and Ia membrane glycoproteins have been initiated.

Pa-2 is the major murine T cell specific mitogen isolated from pokeweed. The carbohydrate specificity of this lectin was unknown and it was not clear whether Pa-2 (and the other pokeweed mitogens) actually bound to the carbohydrate portion of glycoproteins or to some other determinant. Several attempts to identify a mono-, di-, or trisaccharide which specifically bound to Pa-2 were unsuccessful. A clue to the possible specificity of Pa-2 was obtained when preparations of bacterial C-poly-saccharide were found to inhibit hemagglutination by Pa-2. Further investigation with long chain polysaccharides indicated that Pa-2 binds to oligomeric and polymeric N-acetyl-D-glucosamine. Pa-2 does not bind to the monomer, and binds only weakly to the dimer and trimer. These results suggest that the carbohydrate binding site on Pa-2 is quite large and that these must be multiple points of interaction between this protein and the carbohydrate in order to achieve sufficient total binding energy to stabilize the complex. While it appears that N-Acetyl-D-Glucosamine is required for efficient binding, these results do not preclude the possibility that adjacent residues of another saccharide would help to stabilize binding. This latter mechanism would seem to be important in the binding of of Pa-2 to glycoproteins.

We have previously reported preliminary data which indicated that several lectins bind to the same major set of acceptors on murine lymphocytes. These data have been extended to 9 lectins and further evidence obtained from serial depletion studies all corroborate our initial findings. The major cell surface acceptors from murine T and B lymphocytes have been radioactively labeled, isolated by affinity chromatography and SDS-polyacrylamide gel electrophoresis and identified by specific immune precipitation. The acceptor glycoproteins, which bind mitogenic as well as nonmitogenic lectins, isolated from murine T cells are products of the H-2K and D genes, products of the Ia (I region associated) genes and the theta antigen. Acceptors from murine B cells include IgM, IgD, H-2K, H-2D, and the Ia antigens. In addition, glycolipids on both T and B cells bind to the lectins. Preliminary experiments with human lymphocytes (both normal and hyperplastic) give essentially the same patterns as murine lymphocytes.

Attempts to block mitogen activities by capping or blocking the acceptors with antibodies have been unsuccessful. This suggests that either (1) activation is not mediated by a specific receptor, but by a general mechanism which may involve several possible acceptors, or that (2) the specific receptor(s) have yet to be identified.

The existance of a specific receptor for mitogen activation is unlikely in view of our studies on the chemical modification of mitogens. These results substantiate the viewpoint that the B and T cell specificities of plant mitogens are not simply determined by binding to different acceptors or specific receptors on the two cell types. Rather, a more complex set of further interactions, such as the cross-linking of close versus relatively distant acceptors, plays an important role in activating or paralysing B and T cells. Certain T cell mitogens may inhibit mitoses of B cells, probably

by causing an extensive local cross-linking of their acceptors. If this local cross-linking is reduced and distant cross-linking is enabled by chemical modification of the degree of polymerization or aggregation of the mitogen, then B cells can be stimulated by modified mitogens.

Human lymphocytes respond somewhat differently to stimulation by the pokeweed mitogens than do murine lymphocytes. Human B cells proliferate weakly after treatment with Pa-1, while the B cells from nude mouse or ATXBM mous spleens respond vigorously. But if human T cells are mixed with B cells then the B cells proliferate in response to Pa-1 and differentiate into immunoglobulin. producing cells. Murine B cells, either alone or mixed with T cells, do not respond significantly to Pa-2, and in fact both proliferation and immunoglobulin synthesis are suppressed by Pa-2. However, the B cells in mixtures of human B+T cells both proliferate and produce immunoglobulin in response to Pa-2 stimulation. In this case maximum immunoglobulin production occurs at lower mitogen concentrations than does maximum proliferation.

Although Pa-1 only weakly stimulates human B cells to proliferate and produce immunoglobulin in the absence of T cells, it is superior to LPS as a stimulant. Both Pa-1 and Pa-2 are better stimulators of human B cells in B+T cell mixtures than LPS or any other mitogen thus far tested. The role of the T cell in the activation of human B cells by Pa-1 and Pa-2 is currently being pursued, as are further studies in the interaction between mitogens and the cell surface.

Canine free secretory component (FSC; MW 77,500 Daltons) has been previously isolated and characterized in this laboratory. FSC was cleaved with cyanogen bromide and two major fragments (MW 55,000 and 17,000 Daltons) were isolated. Chemical characterization of these fragments indicated that only the larger one was composed of several smaller fragments held together by disulfide bonds.

Similar fragments were isolated from the bound secretory component attached to secretory IgA (sIgA) after cyanogen bromide cleavage. In addition, J chain was also released from sIgA. These data suggest that in canine sIgA, like in human sIgA, the major cyanogen bromide fragments of bound secretory piece and J chain are linked to different fragments of the α chain and are not mutually connected by disulfide bonds. Studies are now under way to determine the position of the disulfide bonds joining secretory component to the α chain in sIgA.

Amyloid proteins of unknown origin are a class of pathological amyloid fibrils deposited in a form of systemic amyloidosis distinct from human amyloidosis of immunoglobulin origin. One of the major proteins solubilized from these deposits cross-reacts with antibodies to human prealbumin. We have established a micro-sequencing technology, using [^{35}S] PITC and autoradiography, to study this and other proteins. Preliminary sequence studies show both similarities and differences between prealbumin and one of the amyloid proteins. Future studies will include sequence analysis of other amyloid proteins and of serum proteins which are antigenically related to them.

The Laboratory of Immunology has a long standing interest in the genetics serology and chemistry of the products of the major histocompatibility complex (MHC) of the guinea pig. This year, an effort to obtin data on the primary amino acid sequence of the B and I antigens was initiated. Guinea pig lymph node cells were cultured in a medium containing a mixture of 10 tritiated amino acids of high specific activity. The cells were then washed, lysed in the non-conic detergent NP-40, ultracentrifuged and partially purified by passage over a lentil lectin column. The eluted material was specifically precipitated with alloantiserum, using the Staph. aureus, Cowan I technique. The precipitate was dissolved in sodium dodecyl sulfate (SDS) and electrophoresed on SDS-polyacrylamide gels. The purified radiolabeled glycoproteins were placed on the automatic sequencer and the radioactive amino acid identified by high pressure and/or thin layer chromatography. Antigen B.1 was determined to have the following sequence for the first 15 amino acids from the N-terminus: 1- not identified; 2- not identified; 3-histidine; 4- not identified; 5- leucine; 6- arginine; 7- tyrosine; 8- phenylalanine; 9- tyrosine; 10- not identified; 11- alanine; 12- valine; 13- not identified; 14- not identified; 15- proline. At six of the 9 positions at which amino acids were identified, the same amino acid has been found in the comparable mouse (H-2D and/or K) and human (HLA-A and/or B) proteins. This indicates a high order of evolutionary homology in these membrane glycoproteins. Continued studies on these molecules should provide information on the chemical basis for the varied and important functions of the MHC gene products.

PUBLICATIONS:

Thompson, R.E., Reynolds, H.Y. and Waxdal, M. N.: Structural composition of canine secretory component and immunoglobulin A. Biochemistry, 14: 2853-2859, 1975.

Waxdal, M.J., Nilsson, S.F. and Basham, T.Y.: Heterogeneity of the pokeweed mitogens and the responding lymphocytes. In The Role of Mitogens in Immunobiology, ed. Oppenheim, J.J. and Rosenstreich, D., Academic Press, N.Y. In press.

Nilsson, S.F. and Waxdal, M.J.: Isolation and identification of the major Concanavalin A binding glycoproteins from murine lymphocytes. Biochem., in press.

Janossy, G., Gomez de la Concha, E., Waxdal, M.J. and Platts-Mills, T.: The effect of purified mitogenic proteins (Pa-1 and Pa-2) from pokeweed on human T and B lymphocytes in vitro. Clin. Exp. Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00040-02 LI

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)
GENETIC CONTROL OF IMMUNOCOMPETENT CELL INTERACTIONS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	5.0	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

Summary

The long range goal of these experiments is to determine the function of the immune response (Ir) genes in the regulation of the immune response and in the control of immunocompetent cell interactions. At present the main areas of investigation include: 1) serological and genetic studies of the antigens of the guinea pig major histocompatibility complex. 2) Physico-chemical characterization of immune response associated (Ia) antigens. 3) The role of the Ia antigens and Ir gene products in macrophage-thumus derived lymphocyte interactions.

Introduction

The major histocompatibility comples (MHC) contains genes which control several important functions related to the immune system including the acceptance and rejection of allografts, the regulation of interactions between immunocompetent cells, the regulation of the ability to mount specific immune responses, and the synthesis of several of the components of the complement system. The MHC also contains genes which encode cell surface glycoproteins which act as histocompatibility antigens. One group of these antigens, the I-region associated (Ia) antigens, are closely linked to the specific immune response (Ir) genes and anti-Ia sera are valuable tools in the dissection of the function of Ir gene products. Several years ago we demonstrated in the guinea pig that Ir gene controlled T lymphocyte response could be inhibited by alloantisera in a haplotype specific manner. This observation suggested the likelihood of intimate associations of Ir gene product function and MHC alloantigens and led us to a detailed study of the genetics, serology, chemistry and function of guinea pig MHC antigens.

Serologic and Genetic Studies of the Guinea Pig MHC Antigens

These studies have been carried out in close collaboration with Dr. A. Geczy and Dr. A. deWeck, Institute for Clinical Immunology, Bern, Switzerland. At least two distinct regions have been identified in the guinea pig MHC (the GPLA complex). Antisera to antigens of the B-region have been primarily raised by cross-immunization of random-bred animals; this region controls the antigens B.1, B.2, B.3 and B.4 which behave as alleles at a single locus and which resemble the products of the murine D or K region genes in their tissue distribution and molecular characteristics. Cross immunization of inbred strain 2 and strain 13 animals, both of which possess the B.1 antigen, leads to sera which identify antigens which resemble the products of the I region of the murine MHC. The availability of a number of inbred strains and families has allowed us to define at least 7 specific Ia antigens. Specific absorption experiments have shown that 2 anti-13 serum contains antibodies to two distinct Ia antigens (Ia.1 and Ia.3), while 13 anti-2 serum contains antibodies to two other distinct antigens, termed Ia.2 and Ia.4. Recently, we have identified a number of Ia antigens which are present on both strain 2 and strain 13 lymphoid cells. Functionally monospecific sera have been prepared in the past year to most of the individual Ia specificities.

Biochemical Characterization of Guinea Pig Histocompatibility Antigens

Because of the crucial role which MHC gene products play in the control of the immune response, the chemistry and structure of these antigens were examined. The techniques include labeling the cells internally with ^{3}H-leucine or ^{3}H-fucose, detergent solubilization of the cell, purification by affinity chromatography using lentil lectin, specific immunoprecipitation, and analysis by electrophoresis on SDS-polyacrylamide gels. One of the difficulties in the genetic mapping of the Ia determinants in the guinea pig system is the lack of strains of guinea pigs bearing recombinant GPLA gene complexes. This difficulty has been overcome in part through the use of sequential immuno-

precipitation procedures which allows a determination of whether two antigenic determinants are on the same or different molecules (i.e. - are encoded by the same or different genes). We have demonstrated that in strain 2 guinea pigs, Ia.2 and Ia.4 are found independent molecules and are most likely the products of separate loci within the I-region. We have also demonstrated that Ia.3 and Ia.5 are on the same molecule in strain 13 animals and that Ia.4 and Ia.5 are on the same molecule in strain 2 animals.

The relationship between the Ia determinant and the Ir gene product is still not established. It is therefore of great interest that Ia antigens detected with functionally monospecific sera appear to have different physicochemical properties. Thus, under non-reducing conditions Ia.4 and Ia.5 derived from strain 2 cells migrate as molecules of $\sim$ 58,000 daltons, while Ia.2 migrates as two components of $\sim$ 33,000 and $\sim$ 25,000 daltons, respectively. Under non-reducing conditions Ia.3 and Ia.5 from strain 13 cells migrate as two components of $\sim$ 33,000 daltons and $\sim$ 25,000 daltons. In contrast, the Ia.1 antigen, migrated as a single component of $\sim$ 25,000 daltons. The significance of the different chemical profiles for each of the Ia antigens and the genetic basis for the two chain structure of some may have initially been part of a single large chain, and thus encoded within a single I region locus. Enzymatic proteolysis might have cleaved the protein, converting intra-chain to inter-chain disulfide bonds or leaving two chains in non-covalent association. An alternative theory is that each chain is encoded in distinct, possibly unlinked genetic regions; the chains might then be joined either during or after translation. OUr goal in these studies is to continue to investigate the functional, genetic and chemical basis for the association of the Ia antigens and Ir gene products.

Role of Guinea Pig MHC Antigens in Immunocompetent Cell Interactions

In addition to the critical role of Ia antigens in T cell activation, we have previously demonstrated that the activation of immune T lymphocyte proliferation by antigen-pulsed macrophages requires that macrophage and T cell be syngeneic or semi-syngeneic. Experiments using combinations of macrophages and lymphocytes that differed by either GPLA-B or Ia antigens demonstrated that the Ia antigens rather than the B antigens were important in macrophage-T lymphocyte interaction. Further analysis of the role of Ia antigens and Ir gene products in macrophage-T interaction demonstrated that the interaction of macrophage and primed T cell is highly specific and requires identity between macrophage and T cell at the specific Ia antigen associated with the Ir gene product whose function is being examined. Activation does not occur even if macrophage and T cell share a portion of the Ia antigens specified in the same haplotype as long as the macrophage donor lacks the Ir gene under study.

One possible explanation for the histocompatibility restriction observed in the interaction of antigen-pulsed macrophage and immune T cell is that the T cell is incapable of recognizing "native" antigen and can only be sensitized to antigen complexed to "self" proteins. Underthese conditions, the failure of interaction between allogeneic macrophage and T cell can be regarded as a

failure of recognition rather than interaction. Our genetic studies suggest that the "self" antigens appear to be the products of the I-region of the guinea pig MHC.

In order to test this "altered self" hypothesis we have developed an assay for the generation of an in vitro primary response against soluble protein antigens in which non-immune guinea pig T cells can be sensitized and subsequently challenged in tissue culture with antigen-pulsed macrophages. Antigen specific T cell activation as measured by increased DNA synthesis occurred when syngeneic antigen-pulsed macrophages were used for both initial sensitization and secondary challenge. When allogeneic antigen-pulsed macrophages were used in both primary and secondary cultures it was difficult to assess antigen specific stimulation due to the substantial mixed lymphocyte reaction which ensued. However, when T cells from F_1 animals were primed with parental antigen-pulsed macrophages they responded only to the parental macrophage used for initial sensitization, but not to those of the other parent. These findings suggest that T cells are incapable of recognizing "native" antigen and can only be sensitized to altered self determinants which may include modified Ia antigens. We were able to directly evaluate the role of the Ia antigens in the induction of the primary immune response because when macrophages were incubated with alloantisera directed against Ia antigen, T cells could be sensitized to the antisera modified Ia determinants. Anti-Ia treated syngeneic macrophages elicited specific T cell activation to the antisera modified determinants. Similarly, anti-Ia treated allogeneic macrophages also specifically stimulated T cells specific for modified Ia determinants. These studies suggest a strong role for the Ia antigens themselves in the generation of new antigenic determinants. The chemical basis for the interaction of antigen with Ia antigens in the macrophage is at present under study.

PUBLICATIONS

Shevach, E.M., Lee, L., Ben-Sasson, S.Z. Genetic control of macrophage-T lymphocyte interaction. In Rosenthal, A. (Ed): Immune Recognition, Proceedings of 9th Leukocyte Culture Conference, New York, Academic Press, 1975, pp. 627-649.

Bloom, B.R., Stoner, G., Gaffney, J., Shevach, E. and Green, I. Production of migration inhibitory factor and lymphotoxin by non-T cells. Eur. J. Immunol. 5: 220-223, 1975.

Shevach, E., Ben-Sasson, S.Z., Finkelman, F., Paul, W.E. and Green, I. Mechanisms of action of genes controlling the immune response. Trans. Proc. 7: 141-147, 1975.

Rosenthal, A.S., Lipsky, P.E., Shevach, E.M. Macrophage-lymphocyte interaction and antigen-recognition. Federation Proceedings 34: 1743-1748, 1975.

Bloom, B.R. and Shevach, E.M. Requirement for T cells in the production of migration inhibitory factor. J. Exp. Med. 142: 1306-1311, 1975.

Rühl, H. and Shevach, E.M. The effect of alloantisera on antigen-induced T cell proliferation. J. Immunol. 115: 1493-1499, 1975.

Geczy, A.F., deWeck, A.L., Schwartz, B.D. and Shevach, E.M. The major histocompatibility complex of the guinea pig. I. Serologic and genetic studies. J. Immunol. 115: 1704-1710, 1975.

Schwartz, B.D., Kask, A.M., Paul, W.E. and Shevach, E.M. Structural characteristics of the alloantigens determined by the major histocompatibility complex of the guinea pig. J. Exp. Med. 143: 541-558, 1976.

Rosenthal, A.S., Lipsky, P.E. and Shevach, E.M. Macrophage lymphocyte interaction: Morphologic and Functional Correlates. In Van Furth (Ed): Mononuclear Phagocytes in Immunity, Infection, and Pathology, Blackwell Scientific, Oxford, 1975, pp. 813-825.

Shevach, E.M. The role of the macrophage in genetic control of the immune response. Fed. Proceedings. In press.

Rosenthal, A. and Shevach, E. The function of macrophages in T lymphocyte antigen recognition. Contemporary Topics in Immunobiology, in press.

Rosenthal, A. and Shevach, E. Macrophage-T lymphocyte Interaction: The Cellular Basis for Genetic Control of Antigen Recognition. In Katz, D.H. and Benacerraf, B. (Eds.). The Role of the Products of the Histocompatibility Gene Complex in Immune Responses. Academic Press, New York, pp. 335-350.

Schwartz, B.D., Paul, W.E. and Shevach, E.M. Guinea pig Ia antigens: Functional significance and chemical characterization. Trans. Rev. In press.

Shevach, E.M. The function of macrophages in antigen recognition by guinea pig T lymphocytes. III. Genetic analysis of the antigens mediating macrophage T lymphocyte interaction. J. Immunol. 116: 1482-1489, 1976.

Schwartz, B.D., Kask, A.M., Paul, W.E. and Shevach, E.M. Structural characteristics of guinea pig major histocompatibility complex alloantigens. Proc. Tenth Leukocyte Culture Conf. In press.

Schwartz, B.D. and Cullen, S.E. The chemistry of mouse andzguinea pig Ia antigens. In Katz, D.H and Benacerraf, B. (Eds.) The Role of the Products of the Histocompatibility Gene Complex in Immune Responses. Academic Press, N.Y., pp. 691-701.

Cullen, S.E. and Schwartz, B.D. An improved method for isolation of H-2 and Ia alloantigens with immunoprecipitation induced by protein A-bearing staphylococci. J. Immunol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00147-01 LI

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Mechanism of Activation of Thymus-Derived Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to understand at the biochemical level how thymus-derived (T) lymphocytes recognize and respond to antigens. OUr approach has been to develop a murine T lymphocyte proliferation assay so that we could bring to bear on this problem the extensive knowledge that exists on the genetics and serology of the mouse major histocompatibility complex (MHC), a region which codes for gene products involved in many immunological functions. During the past ye r we have demonstrated that the genetic control exerted by immune response (Ir) genes of the MHC is expressed in our assay system and that antibodies directed against I region gene products (Ia antigens) can block the T lymphocyte proliferation. Current work is focusing on the relationship between Ir gene products and the Ia antigens as well as the mechanism by which the antibodies inhibit proliferation.

PHS-6040
(12-75)

The development in our laboratory of a reliable murine T lymphocyte proliferation assay has been a major technical advance in the field of immunology. This technique has allowed us to bring to bear on the problem of how T lymphocytes recognize and respond to antigens the extensive knowledge of mouse genetics and serology. The assay utilizes nylon wool passaged peritoneal exudate cells (PETLES) from immunized mice which are challenged in vitro with the immunizing antigen. Immune responses are measured 5 days later by assessing the incorporation of radiolabeled thymidine into the DNA of dividing cells. We have demonstrated by a number of methods that the proliferating cells are mainly T lymphocytes. Thus the assay could be used to investigate the expression of immune response genes in a system devoid of B lymphocyte participation. We studied seven different antigens, the antibody responses to which were known to be controlled by genes located in the I region of the major histocompatibility complex (MHC). In all cases the failure of an inbred strain to make antibodies against an antigen was paralleled by the failure of the T cells from that strain to proliferate in vitro. In addition collaborative studies with Drs. Dorf and Benacerraf from Harvard Medical School on the more complex system of the immune response to poly (Glu^{53} Lys^{36} Phe^{11}), which involves two separate immune response (Ir) genes, demonstrated that both of these genes are required for the expression of a T cell response. We concluded from these studies that the immune response genes cannot be expressed solely in B lymphocytes as had been postulated by several other investigators.

The recent development of antibodies directed against products of the I region, so called anti-Ia antibodies, has allowed immunologists to investigate whether these gene products are involved in immune responses by attempting to block such reactions with the antisera. In collaboration with Drs. David Sachs and C. Garrison Fathman of the National Cancer Institute, we have demonstrated that the stimulator cell in a mixed lymphocyte reaction can be inhibited with anti-Ia sera suggesting that the Ia-bearing molecules are the antigens being recognized in this immune response. More recently, in collaboration with Dr. Sachs of NCI and Dr. Chella David of the Department of Genetics, Washington University School of Medicine, we have shown that anti-Ia antibodies can inhibit the T lymphocyte proliferative response. The inhibition was unique in that antibodies directed against the H-2K and D transplantation antigens did not block. The inhibition was also haplotype specific in that only sera directed against Ia antigens from responder strains could inhibit genetically-controlled antigen responses of T lymphocytes from (responder x nonresponder)F_1 hybrids. These data provide the strongest evidence so far that Ia antigens are determinants on Ir gene products and suggest a key role for these molecules in antigen recognition by T lymphocytes.

Current work is now being directed at the mechanism by which anti-Ia antibodies cause inhibition. Although the assay measures T lymphocyte proliferation, the system also contains macrophages which might function in antigen presentation to the lymphocytes. Therefore, we have undertaken to ascertain whether one or both of these cell types have on their surface Ia antigens which would be blockable with the antisera. Studies on thymocytes carried out in conjunction with Dr. Benjamin Schwartz in this laboratory and, Ms. Susan Sharrow in the Immunology Branch, NCI, have demonstrated small but

detectable amounts of Ia antigens synthesized by thymocyte populations purified of contaminating B cells on the fluorescence-activated cell sorter. At the same time studies on peritoneal exudate macrophages carried out by the same people in conjunction with Drs. David Sachs and Howard Dickler of the Immunology Branch, NCI, have reached similar conclusions i.e. that a small amount of Ia antigens are synthesized by a purified population of macrophages. Thus, we would have to conclude that anti-Ia antibodies might be inhibiting the immunological function of either cell type in the proliferation assay.

Our attempts to understand the mechanism of T lymphocyte activation are of fundamental importance to a basic understanding of how the immune system functions. Since this system plays a critical role in the bodies defense mechanisms against infectious diseases, tumors and transplanted tissues, achieving our goal would hopefully provide major insights into how to manipulate the immune system for the benefit of the patient.

PUBLICATIONS:

Schwartz, R.H., Jackson, L. and Paul, W.E. T Lymphocyte-Enriched Murine Peritoneal Exudate Cells. I. A Reliable Assay For Antigen-Induced T Lymphocyte Proliferation. J. Immunol. 115: 1330-1338, 1975.

Schwartz, R.H., Fathman, C.G. and Sachs, D.H. Inhibition of Stimulation in Murine Mixed Lymphocyte Cultures with an Alloantiserum Directed Against a Shared Ia Determinant. J. Immunol. 116: 929-935, 1976.

Schwartz, R.H. and Paul, W.E. T Lymphocyte-Enriched Murine Peritoneal Exudate Cells. II. Genetic Control of Antigen-Induced T Lymphocyte Proliferation. J. Exp. Med. 143: 529-539, 1976.

Schwartz, R.H., Dorf, M.E., Benacerraf, B. and Paul, W.E. The Requirement For Two Complementing Ir-GLΦ Immune Response Genes in the T-Lymphocyte Proliferative Response to Poly ($Glu^{53}Lys^{36}Phe^{11}$). J. Exp. Med. 143: 897-905, 1976.

Schwartz,R.H., David, C.S., Sachs, D.H. and Paul, W.E. T Lymphocyte-Enriched Murine Peritoneal Exudate Cells. III. Inhibition of Antigen-Induced T Lymphocyte Proliferation with Antihistocompatibility Antisera. J. Immunol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00148-01 LI

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
A RECEPTOR FOR COMPLEMENT IN TISSUES IN IMMUNOPATHOLOGICAL CONDITIONS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Michael Frank, LCI/NIAID
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LAB/BRANCH
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SECTION

INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	--

SUMMARY OF WORK (200 words or less - underline keywords)

There is a receptor for C3 in the human renal glomerulus. Scanning electron microscopic studies demonstrates that this receptor is on the epithelial cell. The presence or absence of this receptor was investigated in a series of patients in whom glomerular C3 was present to a variable extent. In those renal biopsies in which large amounts of C3 were deposited in vivo in the glomeruli, The C3 receptor was not detectable or only minimally defectable, whereas in those patients in whom only small amounts of C3 were deposited in the glomeruli, the C3 receptor was present.

In the course of other studies designed to establish the nature of mononuclear cells in kidney sections from patients with interstitial nephritis it was observed that indicator sheep red blood cells (SRBC) bearing the third component of complement (C3) adhered to the renal glomerulus. This observation suggested that some structure within the glomerulus had a C3 receptor (GCR).

To determine the fine localization of this receptor bacteria were coated with C3. Slices of kidney were then exposed to this reagent and were processed for scanning electromicroscopy. It was observed that the C3 coated bacteria adhered only to the epithelial cell of the glomerulus. These findings suggested that antigen-antibody-complement complexes could be selectively bound to those receptors during immune complex mediated renal disease. If this hypothesis were correct then in those patients in whom a large amount of C3 was deposited in vivo the in vitro activity of the GCR should be diminished.

Renal biopsies from selected patients were therefore studied simultaneously for the presence of the GCR (using 19S EAC). The presence of deposited C3 were detected in frozen sections (using fluorescein labelled anti-C3).

The results of such a study in 23 patients indicated that in those glomeruli in which large amounts of C3 were deposited the GCR was either absent or only minimally detectable. If only small amounts of C3 were deposited then the GCR activity was largely intact. Our interpretation of these results is that the C3 receptor was occupied in vivo and therefore was no longer detectable in vitro. This suggests that this receptor is important in the selective trapping and distribution of C containing complexes in the glomeruli during immune complex renal disease.

Proposed course of the project: The detection of this receptor in an animal model would greatly facilitate the further analysis of the chemical nature of the receptor and its role in experimental immune complex renal disease. Further analysis of relationship between the GCR and the clinical course of a variety of other renal diseases may further clarify the importance of this receptor.

PUBLICATIONS:

Gelfand, M.C., Frank, M.M. and Green, I. A receptor for the third component of complement in the human renal glomerulus. J. Exp. Med. 142: 1029-1036, 1975.

Gelfand, M.C., Shin, M.L., Nagle, R.B., Green, I. and Frank, M.M. The glomerular complement receptor in immunologically mediated renal glomerular injurys. N. Eng. J. of Med. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A100039-01 LI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tumor Immunology in Inbred Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

Project terminated.

PHS-NIH
Rocky Mountain Laboratory
Hamilton, Montana
July 1, 1975 through June 30, 1976

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PHS-NIH
Summary Statement
Rocky Mountain Laboratory
Hamilton, Montana
July 1, 1975 through June 30, 1976

RESEARCH HIGHLIGHTS

During the past year, staff of the laboratory reported their research in 58 publications. An additional 30 are currently in press. Some of the highlights of this research appear in the following sections.

RICKETTSIOSES

Collaborative investigations on the ecology of Rocky Mountain spotted fever (RMsf) were concentrated in New York and Texas. The New York State Health Department was concerned about the prevalence of infected Dermacentor variabilis in Suffolk and Nassau counties and in Long Island, where 36 cases were recorded in 1975. The distribution of human cases was similar to that of infected ticks; infection rates ranged from 0 to 13% (average 7%). The heaviest population was found in grass habitats in the coastal areas. One fatal case in late November was thought to have been due to Rickettsia rickettsii transmitted by Ixodes scapularis.

In Texas, emphasis was placed on ticks removed from dogs owned by RMsf patients. Rhipicephalus sanguineus (358), Amblyomma americanum (151), and D. variabilis (30) were collected and examined, but only the latter had typical R. rickettsii. Of 67 dogs bled, 33% had antibodies to R. rickettsii and 81% had antibodies to R. rhipicephali. These strains were indistinguishable from those isolated last year from R. sanguineus in Alabama and North Carolina. Apparently the organism is widely distributed in the south. The fine structure of this organism is generally typical for spotted fever group rickettsiae, except that it was more pleomorphic. As yet, there is no solid evidence that R. rhipicephali is pathogenic for man, but the serum from one patient thought to have a rickettsial disease was negative to R. rickettsii and positive for R. rhipicephali at 1:32 dilution. (Burgdorfer, Brinton, Krinsky)

Because of the diversity of mammalian hosts and vectors of RMsf rickettsiae, numerous variant types of this organism are found in nature, but little is known about their role in human disease. Hence, it becomes important, on an epidemiological basis, to be able to identify and classify these organisms and the serologic responses they induce. Antisera prepared in mice against 72 strains (67 RMsf- and 5 typhus-group) have been cross-tested by the microimmunofluorescence method. All strains induced homologous titers from 1:256 to 1:32,768. Patterns of cross-reactions

among classified strains correlated with those obtained by toxin neutralization tests. Sixty had patterns of reactivity characteristic for rickettsiae classified as to 10 different species by other procedures. Twelve that had reactivity patterns that did not conform were separated into 7 distinct serologic groups. Four of these groups comprised isolates from R. sanguineus, I. pacificus, D. parumapertus, and D. occidentalis. The R. montana isolates originating from 9 states in western and eastern United States were distinct from other RMsf members. (Philip, Anacker, Casper)

Various parameters of the immune response to vaccines and to infection were investigated. The neutralizing capacity of IgM directed toward Coxiella burnetii was found to be 100-fold greater, on a molar basis, than that of IgG. Primary attacks of typhus (cases in Ethiopia) were accompanied by rises in both IgM and IgG antibodies, whereas the response in Brill's disease (recrudescent attack) was limited to the IgG class. Both primary and recrudescent typhus occurred in the same epidemic. We obtained evidence that the duration of immunity induced by particulate typhus and RMsf vaccines was longer than that afforded by vaccines containing large amounts of soluble antigen. (Ormsbee)

The mouse L cell has been used extensively for cultivating R. rickettsii, but we found that all cells of the parent line did not support comparable growth. By the "glass fragment" technique 93 clones were established and tested for their ability to support growth. These clones could be separated into 5 distinct morphological groups. Of the 93 clones, 6 appeared to support more growth than the rest. Further quantitative tests will be made to confirm original conclusions. (Gerloff)

SYSTEMATICS OF TICKS

The SEM has been used extensively for two major projects the past year - comprehensive reviews of Ixodes in the United States and the genus Rhipicephalus in Africa. By publishing pictorial classification guides, useful criteria for separating species will be made available to scientists working with ticks in these areas. The report on the Ixodes has been completed, with more than 300 SEM photos included. That on the African rhipicephalids is still in progress, but SEM findings so far have helped to clarify the species status of pravus, punctatus, capensis, and oculatus and other groups. (Keirans and Clifford)

ARTHROPOD-BORNE VIRUSES

During the past year, primary attention was directed toward the ecology of viruses carried by argasid ticks, principally species common to the Western Hemisphere. Of 265 pools of ticks examined for viruses, isolates were obtained from 65. Two closely related to Hughes virus were recovered from 36 pools of Ornithodoros capensis collected on Aves Island

in the Caribbean and 63 strains of Soldado virus were isolated from 164 pools of the same species taken from various islands adjacent to Texas. These ticks also yielded 3 isolates of a new virus related to but not identical with Upolo virus. This is an RNA virus 100-220 nm in size, has a lipid outer envelope, and is sensitive to acid and heat. Immunologically, it crosses one way with an ungrouped agent from a related Australian species of Ornithodoros. Unlike Upolo virus, this agent is pathogenic for mice inoculated intraperitoneally. (Yunker, Clifford, Thomas, Keirans)

The role of garter snakes in the ecology of Western equine encephalitis has been difficult to evaluate because the immune response to experimental exposure could not be measured reliably. This problem has been resolved by precipitating virus-antibody complexes (with rabbit anti-snake Ig) before residual virus in neutralization tests is quantitated. We found that garter snakes were equally susceptible to virus given subcutaneously or intraperitoneally, that the magnitude of neither viremia nor the immune response was dose dependent, and that snakes were resistant to reinfection. (Thomas, Coe)

After 12 years of effort, a serially-propagating permanent cell line has been established from D. parumapertus, a common rabbit tick. Before this achievement, cells from only one other species of tick, Rhipicephalus appendiculatus, had been established in culture. Karyological studies of the D. parumapertus line indicate that it is 85% diploid, with fibroblast cells predominating. Preliminary results suggest that this cell line, like primary tick tissue cultures, shows less ability to differentiate biologically between tick- and mosquito-borne viruses than do mosquito cells. The reason for successful cultivation of this cell line and the failure to grow cells of D. andersoni by the same method is not apparent. During the past year, 77 cultures of the latter were initiated, but none survived beyond the 9th passage. (Bhat, Yunker)

In view of the difficulty in cultivating and assaying dengue viruses, additional mosquito cell lines were examined for their utility in this work. We had previously noted that the Aedes aegypti cell line of Peleg occasionally responded to infection by forming syncytia. After cloning this cell line, we found that 3 of 10 clone populations produced more virus and syncytia than Singh's Ae. albopictus cells, but they failed to plaque dengue-2 virus as efficiently. Five other cell lines from Aedes and Culex mosquitos were also inferior to albopictus in virus-plaquing ability. (Yunker)

Because of the prolonged viremia in Colorado tick fever infections in man and its prevalence in the Northwest, the Red Cross might be concerned about this disease in its blood donor program. Last year 60 isolates were obtained from 174 blood samples from persons in Montana, Wyoming, and Idaho. One Hamilton, Montana, resident donated blood 18 hours before onset and the disease was subsequently transmitted to a cancer

patient who received the unit. A survey of serums from 4,000 blood donors revealed that only about 1% had antibodies. Apparently, the hazard is minimal. (Philip, Casper)

RABIES

Continued research on this disease has dealt chiefly with mechanisms involved in abortive rabies and chronic or recrudescent rabies. We have recently found that the usual 10-day period required to abort the disease is shortened when extracts of Bordetella pertussis were added to the inoculum. To date, recrudescent rabies has been produced in one cat and apparent chronic rabies in two others. Lesions in the latter were similar to those of purported chronic human rabies, as reported in the USSR. Temperature sensitive mutants, considered important in the establishment of chronic rabies, were tested for their ability to cause chronic disease in hosts held at high ambient temperature. These viruses varied widely in infectivity, virulence, and incubation period, but so far chronic progressive disease has not been produced. (Bell, Munoz)

PATHOGENIC FUNGI

Studies with several strains of Candida albicans, Group A, have shown a variation in their immunogenicity; some induced higher and more consistent Group A specific antibody in rabbits. Less antigenic strains sometimes induced specific Group A antibody but others did not. The humoral response induced by sublethal doses was unpredictable in rabbits, and there was no correlation between chronic infection and the detection of antibody. (Hasenclever)

PERTUSSIS

Previously, we had succeeded in isolating pure fractions of "pertussigen" (formerly called histamine sensitizing factor) which was composed chiefly of protein and lipid. Because yields were small, further inquiry was made into methods providing larger yields. The best so far developed prescribes treating fresh cells with lysozyme, DNase, RNase, followed by repeated precipitation and passing the final solution through G-200 Sephadex or Biogel columns.

"Pertussigen" is an extremely active adjuvant and is known to enhance the production of hyperacute experimental allergic encephalomyelitis. However, the role of this substance in the pathogenesis of this disease has not been clarified. We showed that increased permeability of the vascular bed of the brain and spinal cord occurred in rats given pertussigen and guinea pig brain, that leakage of radiolabelled human serum albumin in these areas correlated with onset of disease, and that the extent of paralysis was dependent on the amount of leakage from vessels feeding these tissues. Paralysis is thought to be due to an enhanced

reactivity of the vascular bed to vasoactive amines released at the site of antigen-antibody reaction, and not due to direct damage to nerve tissue. (Munoz, Bergman)

IMMUNOLOGY OF VIRAL AND NEOPLASTIC DISEASES

These projects are concerned with both specific and nonspecific mechanisms of immunity and the factors that influence them.

Friend mouse leukemia virus. In continued research on this problem, we found that recovery from leukemia, mediated in part by the Rfv-1 gene located at the D region within the H-2 complex, is also dependent on 3 to 5 other genes derived from the C57BL mouse genome. These genes control development of antibodies directed against the virus and leukemic cells. These antibodies eliminate the virus, even in mice with progressive leukemia. Mice that recover from leukemia produce splenic T lymphocytes that kill leukemic cells, in vitro, independent of macrophages or B lymphocytes. It is thought the Rfv-1 gene may influence the kinetics of production of the cytotoxic T lymphocytes. (Chesebro)

Nonspecific immunity in viral infections. The effects of tuberculin delayed-type hypersensitivity reactions on vaccinia virus (VV) infections in rabbit skin and the nature of protection afforded against encephalomyocarditis (EMC) virus and VV infections by killed mycobacterial cell or cell-wall vaccine (oil in water emulsion) were principally investigated. Tuberculin reactions initiated at sites of primary vaccinia infection reduced viral replication by >90%; these dramatic clinical and virological cures were associated with high titers of acid stable nonimmune interferon, whereas in uninfected skin an immunologically induced acid labile interferon was detected. When 150 μg of BCG cell-wall-oil vaccine was given on one side of a normal unsensitized rabbit, replication of VV was completely inhibited on the contralateral side. This vaccine also protected mice against a lethal challenge of EMC virus for at least 4 months after vaccination. Because 50% of the survivors had no serum neutralizing antibody, part of the protection appeared to be nonspecific. These findings appear to have application to the treatment of acute and persistent viral infections. (Lodmell)

ROLE OF Ig CLASSES IN IMMUNE PHENOMENA

Syrian hamster lymphoid cells have been found to consist of two distinct populations (T and B cells) similar to other mammals and unique T cell antigens have been detected with rabbit anti-thymocyte antisera after absorption with a hamster B cell lymphoma. This SV-40 induced B cell tumor is unusual because it synthesizes a monoclonal γ_2 globulin and, although γ_2 Ig is present in the cell's membrane, antibody to γ_2 will neither cap the membrane Ig nor kill the tumor cell. Male hamsters bearing tumors frequently develop a sex-limited serum and globulin not present in

normal adult males. This protein, found in normal adult females, has been further characterized as 150,000 mw in size and composed of noncovalently linked 25,000 mw subunits. (Coe, Portis)

IMMUNOPOTENTIATION BY MYCOBACTERIAL FRACTIONS

The trehalose mycolates of mycobacteria have been shown to have a critical role in the immunology of tuberculosis and the regression of tumors, but little is known about the mechanisms of action. By proper combination of mineral oil with these mycolates (without any antigen) we prepared emulsions that produced tremendous lung and spleen weights (cellular content). Using an *in vitro* method for measuring chemotaxis, we showed that only those molecules that possess *in vivo* granulomatogenic activity generated chemotactic substances from plasma. Thus, it appears that one of the mechanisms is the generation of chemotactic factors that attract cells to the tissues in which oil droplets are localized. By comparing the activities of structural analogs of mycolic acid esters, we found that the generation of these chemotactic substances was the function of the trehalose moeity. Trehalose alone was not active, and it appears that it must be esterified to a long carbon chain, though not as long and complex as the mycolic acids. (McLaughlin, Kelly, Ribi)

MICROBIAL COMPONENTS IN CANCER IMMUNOTHERAPY

Previously, we have shown conclusively that mycobacteria contain both the immunopotentiating and the antigenic components for regressing experimentally induced tumors in guinea pigs. During the past year, we examined other organisms and their fractions to gain some insight into the nature of these critical components and the method by which they had to be presented to the immune system. Whole, killed *Coxiella burnetii* regressed tumors, as did a weakly toxic endotoxin of the organism when combined with trehalose mycolates. Lipopolysaccharides from mutant gram-negative bacteria, in combination with trehalose mycolate, were highly effective in regressing tumors. Others have suggested that microbial immunostimulants had to be injected directly into tumors to achieve regression, but we found that intradermal injections made between the tumor and the draining lymph node, when followed by excision of the primary tumor, eliminated lymph node metastases in 60 to 90% of the animals. Apparently, microbial antigens of diverse composition, when properly presented to the host to stimulate a cell-mediated response, will regress tumors. Our findings indicate that the use of living microorganisms, which sometimes cause serious allergic and infectious complications in cancer patients, can be successfully circumvented. (Ribi, McLaughlin, Kelly, Smith)

ENDOTOXINS

The discovery that line-10 tumors in guinea pigs are regressed by endotoxins combined with trehalose mycolates has revived interest in this field. Efforts were made to determine the component of this complex lipopolysaccharide that was active in tumor regression. So far, we found that the antitumor effect was negatively correlated with the immunogenicity of the O-antigenic component of endotoxin, that there is no correlation between antitumor effect and endotoxic potency, that transmethylated endotoxoids were highly effective, as was an extract of low toxicity for Coxiella burnetii, and that endotoxins from Re-mutant were among the most active. Currently, attempts are being made to separate the active component from the toxic portion. (Milner, Ribi, Kelly)

PATHOGENESIS OF SLOW VIRAL DISEASES

Additional virologic information acquired during the past year provides a clear picture of the replication of scrapie virus in the natural disease in sheep. It appeared first in the suprapharyngeal lymph node, tonsil, mesenteric-portal nodes, ileum, spleen, and peripheral lymph nodes in 8 of 14 normal yearling sheep. The early appearance in tonsil and suprapharyngeal nodes suggest infection by the oral route. Virus first appeared in the CNS of normal sheep after they were 2 years old. In 3- to 4-year-old sheep affected with the disease, highest concentrations of virus were present in the midbrain, diencephalon, and medulla oblongata. Near the terminal phase, the cerebral cortex contains the greatest amount. (Hadlow, Race)

Because of the prior failure to infect mink by feeding Aleutian disease virus, intranasal inoculation was selected as an alternate simulated natural mode of infection. Patterns of morbidity and appearance of antibody in pastels and sapphires were similar to those seen after intraperitoneal inoculation, but 10-fold greater amounts of virus were required to achieve infection. These data were necessary to design an experimental system for studying the spread of virus that occurs under natural conditions. (Hadlow, Race)

PHS-NIH
Summary Statement
Rocky Mountain Laboratory
Hamilton, Montana
July 1, 1975 through June 30, 1976

ADMINISTRATIVE REPORT

In view of the number of senior staff retirements that will occur in the next few years, several staff conferences were held to allow their participation in program planning for the next decade. Most felt that our research program was too diverse, but these same individuals were generally reluctant to reorient their own programs to focus efforts on a common goal. I felt that our program in virology was too diverse and could be strengthened by recruiting a senior virologist to coordinate this program. I had reserved several positions, which, with additional ones from the Institute, could serve as a package to attract an outstanding virologist. However, management could not support this plan because the Institute lacked the necessary positions and numerous pending changes in management precluded decisions at this time. Hence, these vacancies will be filled with staff fellows until definitive plans can be formulated and adopted.

During the past year, 8 support staff and 2 professional staff were recruited. Dr. Charles McLaughlin, Senior Staff Fellow, and Dr. Yusuke Mizuno, Visiting Fellow, were assigned to the Molecular Biology Section to work with Dr. Ribi on tumor immunotherapy. Dr. Marshall Bloom was transferred to the Laboratory of Biology of Viruses, Bethesda, Md., to receive further training. Dr. John Portis and Dr. Michael Kelly completed their tours of duty as Research Associates, and Dr. Ken-ichi Yamamoto completed his assignment as a Visiting Scientist. Five support staff retired and 2 resigned for personal reasons.

PHS-NIH
Summary Statement
Rocky Mountain Laboratory
Hamilton, Montana
July 1, 1975 through June 30, 1976

HONORS AND AWARDS

The scientific recognition afforded staff of the Rocky Mountain Laboratory is reflected by the honors received and their participation in outside scientific activities.

Awards:

Dr. E. Ribi - DHEW Distinguished Service Award, April 1976
Dr. K. Milner - PHS Commendation Medal, August 1975
Dr. R. N. Philip - PHS Commendation Medal, August 1975
Dr. C. E. Yunker - Sigrid Juselius Foundation Award, Helsinki, June 1975

Editorial Boards of Journals:

Dr. J. J. Munoz - Infection and Immunity
Dr. E. Ribi - Infection and Immunity
Dr. C. M. Clifford - Journal of Medical Entomology
Dr. H. G. Stoenner - American Journal of Veterinary Research

Two additional staff members review manuscripts for the Journal of Immunology, Entomological Society of America, Journal of Parasitology, and Journal of Medical Entomology.

Professional Posts:

Dr. H. F. Hasenclever - Chairman, Standards and Examining Committee; member, American Board of Medical Microbiology
Dr. H. G. Stoenner - Chairman, Leptospirosis Committee, U.S. Animal Health Association
Dr. C. E. Yunker - Member, International Committee on Arctic Arboviruses
Dr. E. Ribi - Chairman, US-Japan Tuberculosis Panel; member, BCG ad hoc Committee, NCI, NIH

Invited Lectures and Participation in Symposia:

The following staff - Drs. Ribi, Burgdorfer, Ormsbee, Philip, Yunker, Milner, Hasenclever, and Kelly - presented scientific papers at 11 meetings or symposia sponsored by international organizations. Also, the following staff - Drs. Bell, Burgdorfer, Chesebro, Hadlow, Kelly, Lodmell, McLaughlin, Ormsbee, Munoz, Hasenclever, Milner, Ribi, Stoenner, and Yunker - presented 32 lectures at universities or participated in national symposia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00061-14 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Natural History of Tick-Borne Rickettsial Agents and Their Public Health Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Burgdorfer	Research Entomologist (Medical)	RML NIAID
OTHER:	L. P. Brinton	Senior Scientist	RML NIAID
	W. L. Krinsky	Research Associate	RML NIAID
	R. N. Philip	Medical Director	RML NIAID
	R. K. Gerloff	Scientist Director	RML NIAID
	R. L. Anacker	Research Microbiologist	RML NIAID
	L. A. Thomas	Research Microbiologist	RML NIAID
	E. A. Casper	Nurse Director	RML NIAID

COOPERATING UNITS (if any) Dr. J. L. Benach, State of New York Dept. of Health; Mr. P. V. Fournier, Texas Dept. of Health Resources; Dr. E. S. Murray, Harvard School of Public Health; Dr. K. A. Keyt, Madigan US Army Medical Center.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	1.6	2.9

SUMMARY OF WORK (200 words or less - underline keywords)

This project pertains to the spotted fever problem in the United States and includes studies related to the ecology, identification, and characterization of Rickettsia rickettsii, and of closely related hitherto undescribed tick-borne rickettsiae in this country. Direction and scientific support are provided to outside agencies (state health departments, public health laboratories, hospitals, physicians, etc.) in establishing educational programs about ticks and tick-borne diseases, in conducting tick/rickettsial surveys, and in training personnel in recently developed diagnostic procedures.

PHS-6040
(12-75)

Project Description:

In cooperation with Dr. J. Benach, State of New York Department of Health, a tick/rickettsia survey was initiated in Long Island where in 1974 31 spotted fever cases, one fatal, were recorded. The main objectives of the study were to determine infection rates in Dermacentor variabilis collected in Suffolk and Nassau Counties, and to establish whether infected ticks are distributed throughout Long Island or occur in highly localized foci. Of 1,644 ticks collected from vegetation, 120 (7.2%) were infected with spotted fever group rickettsiae. Infection rates in several localities varied from 0 to 13.1% with highest rates occurring among ticks in coastal areas.

The largest populations of D. variabilis were in grass habitats, particularly on the upland edges of salt marshes, dunes, and grass upland. Ticks were not nearly as abundant in mixed deciduous woodlands and coastal forests.

Of 352 D. variabilis taken off patients by physicians and hospital personnel, 9 (1.8%) were infected with spotted fever-group rickettsiae. During 1975, 36 cases of RMsf, 2 fatal, occurred in Long Island, the majority of them in coastal areas. One fatal case, late in November, may have been due to Rickettsia rickettsii transmitted by Ixodes scapularis.

Assistance in establishing an educational program about tick-borne rickettsial diseases and in conducting a tick/rickettsia survey in Public Health Region 7 of Texas was requested by the Texas Department of Health Resources. In that area, 9 cases of RMsf, one fatal, were recorded from May through August 1975. Initially, emphasis was placed on ticks removed from dogs associated with human cases. Ten percent of 358 Rhipicephalus sanguineus, 3.3% of 30 D. variabilis, 9.9% of 151 Amblyomma americanum, 66.6% of 3 A. inornata, and none of 3 A. maculatum were positive for spotted fever-group rickettsiae. The only bona fide strain of R. rickettsii came from the infected D. variabilis. All strains examined thus far from R. sanguineus represent R. rhipicephali, a new member of the spotted fever group. The rickettsiae detected in A. americanum and A. inornata thus far have not been identified. Additionally, 2 D. variabilis and 9 A. americanum taken off patients were negative.

One engorged A. americanum nymph taken off a patient shortly after death due to RMsf was negative by hemolymph test, but a strain of R. rickettsii was isolated from its gut content. This finding suggests that the nymphal A. americanum was not the vector of the agent.

The second phase of this project was a serologic survey for spotted fever antibodies in dogs from municipal shelters in Region 7. Of 67 dogs

examined, 22 (32.8%) had antibodies to R. rickettsii and 54 (80.5%) against R. rhipicephali, suggesting a rather wide distribution of these agents, particularly R. rhipicephali, in that area.

Rickettsial isolates from R. sanguineus taken from dogs in Texas were found to be indistinguishable from those of ticks in Mississippi, Alabama, and North Carolina. Because the biologic characteristics of this rickettsia differ from those of the hitherto described spotted fever-group agents, it was regarded a new species, and the name Rickettsia rhipicephali, sp. nov., was proposed in reference to its close association with the brown dog tick, Rhipicephalus sanguineus. As yet, there is no solid evidence that R. rhipicephali is pathogenic to man. However, a convalescent serum of a female patient with a symptomatology (fever, headache, lymphadenopathy) suggestive of a rickettsial illness was negative against R. rickettsii, but positive (1:32) against R. rhipicephali. The development of an eschar at the site of tick bite suggests that the patient was bitten by R. sanguineus.

In Massachusetts, since 1960, all cases of spotted fever, except one, were contracted on Cape Cod or on the nearby offshore islands. In view of this highly focal endemicity, the Harvard School of Public Health initiated a tick/rickettsia survey to identify the ecology of R. rickettsii in these areas. Assistance was requested in examining ticks by the hemolymph test and in identifying by fluorescent antibody procedures the nature of rickettsial agents present. Of 639 D. variabilis collected off vegetation in various localities on Nantucket Island, only one was infected with a spotted fever-group rickettsia, possibly R. rickettsii.

One hundred and eighty-two ticks (132 D. andersoni, 42 D. variabilis, 7 A. americanum, and 1 Haemaphysalis chordeilis) removed from patients in various parts of the country were submitted for examination. Eight specimens were infected with R. rickettsii. In 7 instances, our findings led to treatment of patients before onset of illness, and in one instance to confirmation of RMSF.

Research pertaining to rickettsiae related to but distinct from R. rickettsii and to rickettsia-like microorganisms detected in and isolated from D. andersoni, D. variabilis, A. americanum, A. inornata, and I. scapularis were continued. Their identity and relationship to spotted fever group agents are currently being studied by various investigators of RML's Rickettsial Diseases Section (see Project Nos. 00062-07, 00065-03, and 00066-03).

The meadow vole (Microtus pennsylvanicus) appears to be the animal of choice for the isolation of rickettsiae belonging to the spotted fever and typhus groups. To quantitatively evaluate this rodent's susceptibility

to various species of rickettsiae, duration and concentrations of rickettsemias after injection of low numbers (50 to 100) of rickettsiae were determined by infectivity assays of blood samples in Vero cells (see also Project No. 00066-03). Results obtained thus far with one virulent and 2 nonvirulent strains of R. rickettsii indicate that rickettsemias occur from days 3 through 9 with rickettsial concentrations as high as 1,000 organisms per ml of blood.

During the past year, the following investigators spent limited periods of time at RML to receive training in the application of tick examination techniques: Dr. B. Norment, Mississippi State University, Dr. J. Benach, State of New York Department of Health, Dr. R. Carlstein-Reyes, Lawrence Memorial Hospital, Medford, Massachusetts, Dr. A. Body, University of Cincinnati Medical Center, Dr. H. Hull, EIS Officer, Montana State Department of Health, and Mr. P. Fournier, Texas Department of Health Resources, Austin.

In view of the increasing number of spotted fever cases throughout the nation, more health authorities, including CDC, are requesting assistance and guidance in developing educational programs and in conducting tick/rickettsia surveys to determine within their regions prevalence and distribution of spotted fever foci. Indeed, RML's cooperative efforts along these lines in many states have brought about among the public and the medical profession a new awareness of ticks and their potential role as vectors of disease agents, particularly of R. rickettsii. At the same time, our ecologic investigations suggest the existence in the USA of a far more complex group of tick-borne rickettsiae than previously assumed. Increasing reports of spotted fever-like disease, especially in the southern and southeastern states, indicate that some of these hitherto undescribed rickettsial agents are pathogenic to man.

Because of the possible pathogenicity of R. rhipicephali to man, cooperative studies with the Texas Department of Health Resources will be continued, but emphasis will be placed on epidemiologic and serologic follow-up investigations of patients with suspected tick-borne rickettsial disease.

A tick/rickettsia survey scheduled last year at the US Army's Yakima Firing Center in central Washington did not materialize because of the Army's cutback in research funds. With funds now available, Capt. Keyt, Army Medical Center, Tacoma, Washington, plans to initiate this project in the near future and has asked for research assistance.

Quantitative studies pertaining to the susceptibility of meadow voles to various species of spotted fever-and typhus-group rickettsiae will be continued. A similar project is planned with western cottontails

(Sylvilagus nuttallii) providing that a sufficient number of seronegative specimens can be obtained.

A considerable proportion of research time will be devoted to the identification of rickettsiae related to but distinct from R. rickettsii detected and isolated from several species of ixodid ticks in various parts of the United States.

Guidance and assistance to outside agencies (state health departments, medical research institutions, hospitals, physicians, etc.) in initiating educational programs about ticks and RMsf and in conducting tick/rickettsia surveys will be provided upon request.

RML continued to serve as a WHO Collaborating Centre for Rickettsial Reference and Research in the Western Hemisphere. As such, it provided diagnostic material (antigens, immune sera) and/or strains of viable rickettsial agents to 9 research institutions in the US, Belgium, Egypt, India, and the Honduras.

Publications:

Burgdorfer, W., Sexton, D. J., Gerloff, R. K., Anacker, R. L., Philip, R. N., and Thomas, L. A.: Rhipicephalus sanguineus: vector of a new spotted fever group rickettsia in the United States. Infect. Immun. 12: 205-210, 1975.

Burgdorfer, W.: A review of Rocky Mountain spotted fever (tick-borne typhus), its agent, and its tick vectors in the United States. J. Med. Entomol. 12: 269-278, 1975.

Burgdorfer, W., Adkins, T. R., Jr., and Priester, L. E.: Rocky Mountain spotted fever (tick-borne typhus) in South Carolina: an educational program and tick/rickettsial survey in 1973 and 1974. Am. J. Trop. Med. Hyg. 24: 866-872, 1975.

Sexton, D. J. and Burgdorfer, W.: Clinical and epidemiologic features of Rocky Mountain spotted fever in Mississippi, 1933-1973. South. Med. J. 68: 1529-1535, 1975.

Sexton, D. J., Burgdorfer, W., Thomas, L., and Norment, B. R.: Rocky Mountain spotted fever in Mississippi: survey for spotted fever antibodies in dogs and for spotted fever group rickettsiae in dog ticks. Am. J. Epidemiol. 103: 192-197, 1976.

Publications: (cont'd)

Burgdorfer, W.: Introduction, epidemic (louse-borne) typhus, murine (flea-borne) typhus, Queensland tick typhus, rickettsialpox, trench fever. In Hunter, G. W., Swartzwelder, J. C., and Clyde, D. F. (Eds.): Tropical Medicine. (ed. 5). Philadelphia, W. B. Saunders Co., 1976, sect. II, pp. 95-97, 102-109, 110-111, 122, 123, 135-136.

Burgdorfer, W.: The relapsing fevers. In Hunter, G. W., Swartzwelder, J. C., and Clyde, D. F. (Eds.): Tropical Medicine. (ed. 5). Philadelphia, W. B. Saunders Co., 1976, sect. III, pp. 137-146.

Burgdorfer, W.: The epidemiology of the relapsing fevers. In Johnson, R. C. (Ed.): The Biology of Parasitic Spirochetes. New York, Academic Press, 1976, pp. 191-200.

Burgdorfer, W.: The diagnosis of relapsing fevers. In Johnson, R. C. (Ed.): The Biology of Parasitic Spirochetes. New York, Academic Press, 1976, pp. 225-234.

In press:

Burgdorfer, W.: Current status of tick-borne typhus (Rocky Mountain spotted fever) in the United States. Proc. IV International Congress of Acarology, Saalfelden, Austria, Aug. 12-19, 1974.

Krinsky, W. L., and Burgdorfer, W.: Trypanosomes in Amblyomma americanum from Oklahoma. J. Parasitol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00062-07 RML

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular and Subcellular Aspects of Vector-Pathogen Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. P. Brinton	Senior Scientist	RML NIAID
OTHER:	W. Burgdorfer	Research Entomologist (Medical)	RML NIAID
	W. L. Krinsky	Research Associate	RML NIAID
	W. J. Hadlow	Research Veterinarian (Path.)	RML NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION

Rickettsial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The long term objective of this project is to establish a clearer understanding of the interaction of pathogens of man, especially zoonotic agents, with host cells of vectoring arthropods and their vertebrate hosts. This primarily encompasses cellular and subcellular studies of both in vivo and in vitro material infected with protozoal, bacterial, and viral agents. Studies continuing and/or currently in progress include: 1) Colorado tick fever virus development in Dermacentor andersoni ticks, 2) comparative fine structure of Wolbachia-like organisms in D. andersoni, Rhipicephalus sanguineus and Haemaphysalis leporispalustris ticks, 3) developmental fine structure of gut from normal and Rickettsia rickettsii-infected D. andersoni ticks, 4) comparative fine structure and cytodifferentiation of R. rickettsii and R. rhipicephali in D. andersoni, R. sanguineus and in cell culture, and 5) studies on the fine structure and cytomorphology of the 277F agent belonging to the recently described genus Spiroplasma.

Project Description:

The long term objective of this project is to establish a clearer understanding of the interaction of pathogens of man, especially zoonotic agents, with host cells of vectoring arthropods and their vertebrate hosts. Electron microscopic techniques are applied to characterize subcellular structures of normal and infected arthropod tissues and to determine the cytodifferentiation and cytomorphology of microorganisms during intracellular and/or extracellular development. While the project on Colorado tick fever virus morphogenesis and localization in tissues of Dermacentor andersoni continued to be open, work was temporarily terminated, while efforts were directed toward other projects.

Sufficient data is now available for manuscript preparation concerning the comparative fine structure study on Wolbachia-like symbiotes in D. andersoni, Rhipicephalus sanguineus, and Haemaphysalis leporispalustris ticks. Because a clearly defined cell wall is absent, naturally occurring symbiotes in D. andersoni and H. leporispalustris are mycoplasma-like organisms. Fine structural analysis of symbiotes of H. leporispalustris is difficult because they are susceptible to shrinkage and are sparse at the cell level. The prominent gram-negative type cell walls and general fine structure of symbiotes in R. sanguineus ticks clearly places these organisms in the genus Wolbachia.

The comparative fine structure of Rickettsia rickettsii and R. rhipicephali was studied in both Vero cells and in tissues of D. andersoni and R. sanguineus ticks. R. rhipicephali organisms in R. sanguineus ticks exhibit a morphological fine structure that is generally typical for spotted fever-group rickettsiae, except that they are more pleomorphic. In tick tissues, R. rhipicephali had a maximum length of just under 2.0 µm, but in Vero cells they were longer than 3.0 µm. Their cytoplasm contains variably dispersed, yet well-defined ribosomes in a finely granular amorphous matrix, which also contains a fibrillar reticulum.

Fine structure studies on the cytomorphology of the 277F agent were completed. While this organism lacks a cell wall and axial filaments, it conforms closely in size and fine structural characteristics (plasma membrane, ribosomes, fibrillar reticulum, and thin glycocalyx-like coat on the cell surface) to the genus Spiroplasma of the order Mycoplasmatales.

A purified preparation of seal flipper tumor was examined for virus material utilizing negative staining. Particulate material was observed, yet there was an absence of morphological fine structure, which was probably due to the long term (≃ 20 years) storage of the flipper in formaldehyde. Tentative plans have been made with Dr. Hadlow to initiate an EM study on murine leukemia virus.

A cooperative study on the developmental fine structure of different stages of development of the microsporidian Nosema sp. in Ornithodoros parkeri ticks has been initiated. Preparation of tissues from nymphal and adult ticks are completed for the present.

This project will continue to emphasize developmental fine structure studies on vector-pathogen associations between ticks and zoonotic agents of medical importance. In addition to completing studies on the comparative fine structure of symbiotes in designated tick species and on comparative studies between R. rickettsii and R. rhipicephali, our efforts will be directed toward the following: 1) cellular and subcellular fine structure of Colorado tick fever virus morphogenesis and interaction with host cells and tissues of D. andersoni; 2) gut barrier studies on the passage of R. rickettsii through the intestinal wall of D. andersoni ticks following the blood meal; 3) EM studies of lymphoma-like tumors in mink in cooperation with Dr. W. Hadlow.

Publications:

Krinsky, W. L.: Cystic anomaly in the intestine of adult Tabanidae. J. Invertebr. Pathol. 26: 137-138, 1975.

Burgdorfer, W. and Brinton, L. P.: Mechanisms of transovarial infection of spotted fever rickettsiae in ticks. Ann. N.Y. Acad. Sci. 266: 61-72, 1975.

In press:

Brinton, L. P. and Burgdorfer, W.: The 277F agent: a spiroplasma from the rabbit tick, Haemaphysalis leporispalustris (Acari: Ixodidae). II. Cellular and subcellular organization. J. Bacteriol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00063-06 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immune Responses to Rickettsial Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Ormsbee	Scientist Director	RML NIAID
OTHER:	R. N. Philip	Medical Director	RML NIAID
	E. A. Casper	Nurse Director	RML NIAID

COOPERATING UNITS (if any) Dr. C. L. Wisseman, Jr. and Dr. P. Fiset, Dept. of Microbiology, University of Maryland School of Medicine, Baltimore.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	0.9	1.8

SUMMARY OF WORK (200 words or less - underline keywords)

The general purpose of this project is to study the immune responses in man and animals to natural and experimental rickettsial infections. Presently we are 1) examining the protective effect in mice of human antibodies against Coxiella burnetii, 2) developing new criteria for characterization of recrudescent epidemic typhus fever (Brill-Zinsser disease), and 3) examining the decay of immunity to spotted fever and typhus fever infections in immunized guinea pigs.

Project Description:

The only known function of humoral antibody in resistance to rickettsial infection is opsonization, followed by intracellular destruction in a professional phagocyte. We have attempted to measure the protective effect of specific IgM and IgG human antibodies against Coxiella burnetii in an animal system. The system involves incubating the antibody with live rickettsiae, injecting the mixture into normal mice, and measuring the consequent reduction in rickettsial population by a tissue culture plaque technique. Our results, although preliminary, indicate that IgM antibody is more effective (on a molar basis) than IgG antibody by an order of ≥100. This finding has been made possible by improvements in our tissue culture system in which we can now routinely induce C. burnetii to form numerable plaques in monolayers of primary chick embryo cells in 12 days, 5 days less than formerly.

Recrudescent epidemic typhus (Brill-Zinsser disease) is the primary source of new epidemics of primary epidemic typhus. The true incidence and detection of Brill-Zinsser disease, therefore, is important in the control of outbreaks of primary disease transmitted by lice. By means of a micro-immunofluorescence (MIF) test and highly specific immunologic reagents purified by affinity chromatography, we have been able to identify easily and with certainty cases of Brill-Zinsser disease characterized by rising antibody titers of a purely IgG character. Although these cases occurred concurrently with primary epidemic typhus cases in Ethiopia, they were indistinguishable serologically from authentic Brill-Zinsser cases from Yugoslavia. Primary attacks of epidemic typhus fever were invariably accompanied by rises in both IgM and IgG antibodies. With specific reagents, this MIF test may prove to be a significant improvement over other serologic tests for the detection and characterization of human antibodies against rickettsiae.

Concern has been expressed about the efficacy of commercial vaccines for prophylaxis against spotted fever and epidemic typhus fever. In response to this, we have modified an animal test originally used to assess the potency of Q fever vaccine to the long-term testing of other rickettsial vaccines. Commercially prepared vaccines, including the DBS reference typhus vaccine as well as experimental vaccines prepared both at RML and at the University of Maryland School of Medicine, are being tested. Vaccinated guinea pigs are being given a standard challenge of live rickettsiae 3 weeks, 3 months, 6 months, and 12 months after immunization. Our results to date indicate that the protection afforded by vaccines containing significant amounts of soluble antigen decreased markedly within 3 months whereas resistance induced by vaccines composed of whole rickettsiae remained intact. By 6 months, however, significant diminution of protection occurred even in guinea pigs which had been given the particulate (whole rickettsia) vaccine.

It is planned to continue our study of the immune responses to rickettsial infections and rickettsial antigens with particular emphasis on C. burnetii and its characteristics which appear to set it apart, antigenically speaking, from other rickettsiae. This will be done in collaboration with colleagues at the University of Maryland School of Medicine. It is hoped that these studies will not only increase our understanding of these diseases but also will lead to improved methods of diagnosis, treatment, and prevention of rickettsial disease.

Publications:

Ormsbee, R. A.: Rickettsiae. In Lennette, E. H., Spaulding, E. H., and Truant, J. P. (Eds.): Manual of Clinical Microbiology. (ed. 2). Washington, D.C., American Society for Microbiology, 1974, pp. 805-815.

Ormsbee, R. A.: American spotted fevers, related spotted fevers and rickettsioses, Q fever. In Hunter, G. W., Swartzwelder, J. C., and Clyde, D. F. (Eds.): Tropical Medicine. (ed. 5). Philadelphia, W. B. Saunders Co., 1976, pp. 112-117, 118-121, 131-134.

In press:

Ormsbee, R. A.: Qualitative requirements and utilization of nutrients: Rickettsiales. In Recheigl, Jr., M. (Ed.): Handbook of Nutrition and Food. Cleveland, CRC Press, Inc.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00064-16 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Phase Conversion in Coxiella burnetii, Causative Agent of Q Fever

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. A. Ormsbee Scientist Director RML NIAID
OTHER: None

COOPERATING UNITS (if any) Dr. P. Fiset, Dept. of Microbiology, University of Maryland School of Medicine, Baltimore.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.1	1.8

SUMMARY OF WORK (200 words or less - underline keywords)

Coxiella burnetii differs from other rickettsiae in that it undergoes a change, phase I→phase II, on cultivation in chick embryos and may undergo the reverse, phase II→phase I, in animals. The phase shift is characterized by loss or gain of phase I antigen and in its pathogenicity for guinea pigs and mice.

Project Description:

Our aim is to study the mechanism of this phase change which may be mutational in character and may, under certain circumstances, be irreversible. We are attempting to produce and isolate specific antibody fractions which will enable us to differentiate between phase I and phase II rickettsiae by immunofluorescence technique.

We have passed 3 clones of C. burnetii in embryonated eggs by both high and low inocula. In one clone gradual conversion to phase II has been accompanied by markedly decreased ability to grow to concentrations high enough to make effective CF antigens. This change in growth characteristics was similar to that shown by a 90th egg passage strain which could not be passed in guinea pigs nor made to revert to phase I. It is possible that the ease with which high egg passage strains formerly were made to revert to phase I by passage in guinea pigs may have been a simple selection process from a rickettsial population which was almost entirely but not quite all in phase II.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00065-03 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Antigens and Classification of the Rickettsiae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Anacker	Research Microbiologist	RML NIAID
	R. N. Philip	Medical Director	RML NIAID
OTHER:	W. Burgdorfer	Research Entomologist (Medical)	RML NIAID
	E. A. Casper	Nurse Director	RML NIAID
	R. K. Gerloff	Scientist Director	RML NIAID
	R. F. Smith	Research Chemist (Biochem.)	RML NIAID
	L. A. Thomas	Research Microbiologist	RML NIAID

COOPERATING UNITS (if any) Dr. M. Hamilton, Dept. of Mathematics, Montana State University, Bozeman.

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Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.1	2.4

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objectives of this project are to develop practicable procedures for the classification of spotted fever- and typhus-group rickettsiae and to determine the nature and biological properties of rickettsial antigens and constituents. Current specific interests center on 1) the evaluation of a microimmunofluorescence test for assaying type-specific antibody responses to rickettsial infection in laboratory mice, 2) DNA composition of members of the spotted fever group, and 3) isolation and characterization of rickettsial antigens.

Project Description:

A variety of serological and physicochemical approaches is being utilized to classify rickettsiae within the spotted fever and typhus groups and to isolate and characterize the antigens of these rickettsiae.

Antisera against 72 strains of rickettsiae (67 spotted fever group, 5 typhus group) have now been cross-tested by microimmunofluorescence (MIF). Included are strains which had been classified as to species by other methods, as well as uncharacterized isolates obtained by RML investigators from varied sources in the United States and overseas. Results of these tests indicate rickettsiae can be serologically grouped by this test. Some specific observations include:

1) All strains induced homologous antibodies in weanling mice ranging in titer from 1:256 to 1:32,768. Most sera had titers of 1:512 to 1:2048.

2) Distinct serologic groups were detected. Strains included within groups were homogeneous both as regards reactivity to each other and degree of crossing with strains in heterologous groups.

3) Patterns of cross-reaction among classified strains correlated well with those obtained by Dr. E. J. Bell with the toxin neutralization test (correlation coefficient, r = 0.88). It is noteworthy that antibodies being measured in the 2 tests come from different species of laboratory animals.

4) Sixty strains had patterns of reactivity characteristic for rickettsiae classified as to species by other procedures. Included were Rickettsia rickettsii (29 strains), R. montana (15 strains), R. conorii (5 strains), R. parkeri (2 strains), R. akari (2 strains), R. sibirica (1 strain), R. australis (1 strain), R. prowazekii (2 strains), R. typhi (2 strains), and R. canada (1 strain).

5) Twelve strains had patterns of reactivity that did not conform to those of classified strains. They comprised 7 distinct serologic groups. The fact that 4 groups contain 2 or more strains originating from diverse geographic areas suggests that they are distinct serotypes hitherto unrecognized. The 4 included 1) rickettsiae associated with Rhipicephalus sanguineus recently described by Burgdorfer as a new species of spotted fever-group rickettsiae, 2) 3 strains of spotted fever-like rickettsiae recovered from Ixodes pacificus ticks in Oregon that were recently described by Hughes et al., 3) 3 strains of spotted fever-group rickettsiae recovered by C. B. Philip and H. G. Stoenner from Dermacentor parumapertus ticks from Nevada and Utah, and 4) 2 spotted fever-group rickettsiae isolated by E. J. Bell from D. occidentalis ticks collected in California.

6) The R. montana isolates were sharply different from other members of the spotted fever group. Representatives of this serotype originated from 9 states in both eastern and western United States, thus indicating an ubiquitous distribution.

Two different approaches are being used in attempts to detect differences in rickettsial strains at the subcellular level. First, viable and formalin-killed rickettsiae, purified by renografin and sucrose density gradient centrifugation, respectively, were treated with a variety of extractants or ultrasound. Sonic, ether, and detergent extracts of viable R. rickettsii contained several antigens as revealed in immunodiffusion tests and also protected mice against challenge with the toxin of R. rickettsii. Antigens were obtained from formalin-inactivated rickettsiae with much greater difficulty so those attempts were discontinued.

Electrophoresis of detergent extracts of R. rickettsii and the R. sanguineus agent in agarose containing antibody to R. rickettsii indicated a close relationship between these rickettsiae. Of the 9 antigens found in the R. rickettsii extract, 7 were shared by the R. sanguineus agent.

For the second approach, experiments are in progress to determine the guanine-cytosine content of DNA from a number of rickettsial isolates belonging to the spotted fever group but differing from R. rickettsii in certain properties such as tick host and virulence. Because of technical difficulties with our new spectrophotometer, only a limited number of acceptable analyses have been made. However, our results to date suggest that R. rickettsii (R strain), an eastern Montana member of the spotted fever group (strain 275 F), the R. sanguineus agent (strain 3-7♀6), and an isolate from Amblyomma americanum (strain WB8-2) would be classified together on the basis of their DNA compositions.

Experiments to determine the reliability of a newly designed protection test to evaluate spotted fever vaccines were completed. Areas under the fever curves of guinea pigs, inoculated with vaccine over a restricted dose range and infected with a standardized dose of R. rickettsii, varied linearly with $\log_{10}$ dose. Vaccines were compared on the basis of the dose (50% protective dose) at the midpoint of the linear portion of the dose response. Statistical analysis of results of duplicate tests of the same vaccines indicated that differences between experiments were not significant. This test offers several advantages over the test officially approved for vaccine assay.

Knowledge of antigenic relationships among spotted fever-group rickettsiae has accrued slowly. For many years differentiation of rickettsiae has been based on cross-immunity and vaccine protection tests

in guinea pigs, cross-block CF tests or CF tests with washed species-specific antigens, or mouse toxin neutralization tests. All of these procedures have shortcomings as methods for antigenic classification of rickettsiae. Accumulation of rickettsial isolates as yet antigenically uncharacterized has re-emphasized the need for a practical, universally applicable means for serologic classification and improved methods for antigenic characterization of strains. Such information obviously has epidemiologic as well as taxonomic implications.

Current research activities will be continued in the coming year. Efforts will continue to be directed toward the development of a procedure for classifying rickettsial isolates by MIF tests. Also, guanine-cytosine content of DNA from additional representatives of the various serological subgroups will be determined. Finally, attempts will be continued to isolate protective and species-specific antigens from these rickettsiae.

Publications:

Anacker, R. L., Gerloff, R. K., Thomas, L. A., Mann, R. E., and Bickel, W. D.: Immunological properties of Rickettsia rickettsii purified by zonal centrifugation. Infect. Immun. 11: 1203-1209, 1975.

In press:

Anacker, R. L. and Ormsbee, R. A.: Rickettsiae - general descriptions. In Virology and Rickettsiology, Handbook Series in Clinical Laboratory Science. Cleveland, CRC Press, Inc.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00066-03 RML

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Growth Characteristics of Rickettsiae in Cell Cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. K. Gerloff	Scientist Director	RML NIAID
OTHER:	R. L. Anacker	Research Microbiologist	RML NIAID
	W. Burgdorfer	Research Entomologist (Medical)	RML NIAID
	R. N. Philip	Medical Director	RML NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The broad objective of the project is to study growth characteristics of rickettsiae in cell cultures. In collaboration with other investigators, data obtained are applied to differentiation and classification of rickettsiae, their routine production for serologic, immunologic, and biochemical studies, and studies on their maintenance in nature. Current emphasis is on strains of the spotted fever group and certain unclassified strains, particularly those which grow poorly in embryonated eggs. Standard tissue culture procedures are used, including infectivity titrations by the plaque method. Inocula are frequently centrifuged onto cell monolayers when infection can be enhanced thereby. Different host cells and environmental conditions are tested to find combinations which provide optimum rickettsial growth. The project also includes preparation of purified and semi-purified concentrates of cell culture-grown rickettsiae for use as antigen and development of methods for such purification.

Project Description:

The objectives are 1) to study the ability of various kinds of tissue cultures to support the growth of rickettsiae, 2) to determine optimal conditions for propagating rickettsiae in each kind of tissue culture for the purpose of both obtaining maximum yields and finding features which might aid differentiation and classification of rickettsiae, 3) to produce and, in some instances, purify cell culture-grown rickettsiae in sufficient quantity for use in serological, immunological, and biochemical studies, and 4) to use tissue culture procedures in studying the role of various animals and arthropods in the maintenance and transmission of Rickettsia rickettsii in nature.

Standard procedures common to tissue culture and rickettsiology are used, including 2 methods for titrating infectivity. One utilizes centrifugation of rickettsiae onto cell monolayers to enhance infection; the other involves formation of plaques in monolayers. Considerable use is also made of suspended cell cultures.

1. Cell cloning. Because cells of the parent mouse L line appeared to differ in their ability to support the growth of R. rickettsii, R strain, a study was conducted to see if certain clones would be better hosts for this organism than the parent line. With the "glass fragment" technique 93 clones were established. Samples were simultaneously preserved (liquid N_2) and tested with standardized seed suspension of the rickettsia. The clones could be divided into 5 rather distinct morphological types. Rickettsial TC ID_{50}'s obtained with the various clones did not vary significantly but 6 clones were selected in which the rickettsia grew especially prolifically, as judged from stained smears. Other tests (yield of purified, dry rickettsiae and plaque titrations) are in progress to determine whether the initial conclusions were valid.

2. Collaboration with Dr. Anacker. Production of rickettsiae in tissue cultures for use in Dr. Anacker's studies on DNA and antigenic comparisons has been continued. Major emphasis has been on production of 2 strains of R. rickettsii and Rickettsia rhipicephali (proposed name for new rickettsia from Rhipicephalus sanguineus ticks), with a lesser amount on production of the agent of Kenya tick typhus and a rickettsia isolated from Dermacentor parumapertus ticks. (See Project No. 00065-03 for results obtained.)

3. Collaboration with Dr. Burgdorfer. Studies are being conducted to determine the degree and duration of rickettsemia in meadow voles (Microtus pennsylvanicus) following intraperitoneal injection of low levels of R. rickettsii of different strains. Infectivity assays to date have been done by the plaque method in Vero cells. Ancillary studies are in

progress to determine whether L cells might be a more sensitive test tool. Preliminary results indicate that they are, although plaques in L cells are smaller. (See Project No. 00061-14 for results relative to the voles.)

4. Tests for mycoplasma. Contamination by these organisms is an aspect of tissue culture that can never be ignored. Persons who specialize in study of mycoplasmas contend that bacteriological tests alone are not completely reliable as indicators of cell culture contamination. Thus, an attempt has been made to incorporate a biochemical test into routine screening procedures. The one chosen is based on the fact that mycoplasma-contaminated cells incorporate into their RNA large amounts of radiolabeled uracil (U) but only small amounts of labeled uridine (UdR). The opposite is true of uncontaminated cells. High UdR/U ratios of specific activity in cellular RNA (e.g., 500 to 1200) indicate mycoplasma-free cells; low ratios (e.g., 3 to 10) indicate contamination. Tests to date on our parent L cell line have yielded ratios between 83 and 116; the ratio in a single test of clone 33 was 105. These values are probably in the safe range even though they are lower than those reported by the originators of the test, but further work is needed to verify this.

All of the aforementioned projects, except additional cell cloning, will be continued in the coming year. Because the PI is contemplating retirement, beginning later in the current calendar year, the major emphasis will be to satisfactorily conclude those projects which are now in progress.

Publications:

In press:

Gerloff, R. K.: Tissue cultures and culture media for microorganisms of the tribe Rickettsieae. In Recheigl, Jr., M. (Ed.): Handbook of Nutrition and Food. Cleveland, CRC Press, Inc.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00067-12 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tularemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Bell	Medical Director	RML NIAID
OTHER:	R. N. Philip	Medical Director	RML NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Medical Zoology and Zoonotic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Skin test surveys of native American populations and of other populations potentially exposed to tularemia suggest much higher incidence of the disease than clinical histories would indicate. It is probable that many of the reactors suffered subclinical or undiagnosed tularemia, but antigenic relationships of Francisella tularensis and other organisms (F. novicida, Brucella sp.) pose the possibility that the skin test reaction may not be sufficiently specific for certain diagnosis. Cross reactivity in the skin test between those organisms is being studied with Dr. R. Philip.

Stable quantum differences in susceptibility to tularemia infection and apparent failure to acquire immunity to the organism on enteric exposure have been noted. However, infection acquired via the gastro-intestinal tract is often chronic whereas even one organism, of the strain used, is acutely fatal when introduced parenterally. Effects of population (bacterial) "pressure" in the gut, and of associated flora, are being studied.

Project Description:

Prévalence of tularemia, based on skin test surveys, seems to be much higher in North America, Eurasia, and Japan than reports of clinical cases would indicate. Several explanations can be advanced for the discrepancy, among them subclinical infections from type B F. tularensis, infection by an unknown milder type of organism, repeated antigenic stimulation (e.g., from contaminated water) without infection, or infection by a related organism (F. novicida, Brucella sp., other, unknown) with antigenic relationship. We are inquiring into the specificity of the skin test in laboratory animals infected with F. tularensis, F. novicida, and Brucella abortus.

In tests of infectivity to voles of F. tularensis administered orally, we have noted that mortality at a continuous low level of exposure soon stabilizes, but that additional voles succumb at the next greater level of exposure and again when the concentration of bacteria is increased 10-fold. Thus, there are stable quantum differences in permeability of the gastro-intestinal tract to virulent organisms and apparent dependence of that permeability on population pressure. Moreover, it appears that effective immunity to the organism does not develop in the intestine. We have not yet determined whether IgA is elicited or secreted although the gastro-intestinal route has been used for vaccination against tularemia. The phenomenon of quantum differences in permeability is pertinent to the common occurrence of gram-negative bacteremia and of multifocal liver abscesses.

Many voles infected via the gastro-intestinal tract develop chronic tularemia with nephritis and bacteriuria, even though a single bacterium of the infecting strain introduced parenterally causes acute fatal infection. The ameliorative effect of the enteric route is not explainable by conventional concepts about the pathogenesis of tularemia.

All of the projects above mentioned will be pursued as time permits.

Publications:

Bell, J. F. and Stewart, S. J.: Chronic shedding tularemia nephritis in rodents: possible relation to occurrence of Francisella tularensis in lotic waters. J. Wildl. Dis. 11: 421-430, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00068-16 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Rabies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. F. Bell Medical Director RML NIAID
OTHER: None

COOPERATING UNITS (if any) Dr. J. Munoz, RML; Dr. D. Blenden, Microbiology, University of Missouri, Columbia; Dr. H. F. Clark, Wistar Institute, Philadelphia, Pa.; Dr. D. P. Perl, Neuropathology, Brown Univ. Medical School, Providence, R.I.; Dr. A.M.O. de Diaz and Dr. E. Fuenzelida, Centro Panamer., Buenos Aires.

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Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Medical Zoology and Zoonotic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

There are several separate objectives in our rabies program: 1) work is conducted to trace the rabies vaccine-potentiating activity associated with other characteristics during "pertussigen" purification, 2) the use of skin biopsy as a rabies-diagnostic method during life is being evaluated on aberrant forms of rabies and on preclinical rabies-infected dogs, 3) the effects of high ambient temperature on animals infected with ts mutants of rabies and rabies-related viruses are investigated, 4) use of cerebral spinal fluid neutralization test to monitor the course of chronic CNS virus infection and studies of the histopathology of chronic rabies, and 5) survey to determine the prevalence of abortive rabies in dogs and cats in an enzootic area in Argentina.

Project Description:

Studies of Bordetella pertussis extract (BPE), produced by Dr. Munoz, a few years ago showed that the crude substance is a potent adjuvant for rabies vaccine, inasmuch as antibody titers are higher and resistance to challenge is greater. Also, we have found more recently that the minimal incubation period of 10 days necessary for abortion of rabies infection is shortened when BPE is inoculated with the virus. The immune-adjuvant effect, which may be useful in preventing clinical rabies by accelerating the immune response, is also useful in monitoring purification of the BPE.

The examination of a skin biopsy is increasingly used in rabies diagnosis during life and also for diagnosis of other CNS infections (e.g., herpes). Limitations of the method and significance of presence of antigen in the skin are being explored.

Temperature sensitive (ts) mutants of viruses have been investigated as potentially useful vaccines and are now considered of importance in the establishment of chronic virus infection. Our studies show wide variations among various ts mutants in regard to infectivity, virulence, and incubation period when the hosts are held at high ambient temperature, but thus far no chronic progressive infection has occurred.

Recrudescent rabies has been produced in one cat and apparent chronic rabies in 2 others. Abortive rabies has been produced in several dogs and cats. In each case, the CSF neutralization test has facilitated the monitoring of the course of "infection, not otherwise possible". Lesions produced in the CNS are unlike those of typical acute rabies and similar to those of purported chronic human rabies, as reported in the USSR.

The incidence of abortive rabies in an enzootic area has been found to be very low, although many of the animals had been vaccinated. Therefore, the contention of Bindrich and Schmidt that vaccination is conducive to development of a rabies carrier state, which led to official opposition to vaccination in Germany, cannot be supported. This study is terminated.

Work on adjuvant effects of "pertussigen" is being continued as a tracer for purification and to eliminate a possible effect of endotoxin as a contaminant. Studies with Dr. Blenden on the parameters of the phenomenon of rabies antigen in skin are continuing with emphasis on time of appearance and variation with different strains of virus. Work on effects of high ambient temperature on rabies infection will be directed toward identification of the mechanisms involved. Studies of chronic and recrudescent rabies will be continued (2 cats at hand apparently on

progressive course) to attempt to determine the cause. CSF monitoring will be continued with greater emphasis on blood/brain barrier to cells, proteins, and especially complement (with Dr. Nilsson, U. of Pa.).

Publications:

Bell, J. F.: Latency and abortive rabies. In Baer, G. M. (Ed.): The Natural History of Rabies. New York, Academic Press, Inc., 1975, vol. 1, pp. 331-354.

de Diaz, A.M.O., Fuenzalida, E., and Bell, J. F.: Non-fatal rabies in dogs and cats. Ann. Microbiol. (Paris) 126: 503-509, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00069-45 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Systematics and Vector Relationships of Certain Parasitic Arthropods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Keirans	Research Entomologist (Medical)	RML NIAID
OTHER:	C. M. Clifford	Scientist Director	RML NIAID
	C. E. Yunker	Scientist Director	RML NIAID

COOPERATING UNITS (if any) Dr. H. Hoogstraal, NAMRU-3, Cairo, A.R.E.; Miss Jane B. Walker, Div. of Vet. Services, Onderstepoort, Rep. of South Africa; Dr. F. Lukoschus, Catholic Univ., Nijmegen, Netherlands; Dr. D. Sonenshine, Old Dominion Univ., Norfolk, Va.; Mr. J. Reddell, Texas Tech Univ., Lubbock.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Medical Zoology and Zoonotic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.7	1.7	3.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with investigations into the systematics, including taxonomy and classification, and host-ectoparasite relationships of medically important arthropods, especially ticks and certain mites. The topics of current interest are: 1) a scanning electron microscope study of adults of the genus Ixodes in the United States, 2) computerization of the tick data at RML and NAMRU-3 (Cairo), 3) a long range investigation of the genus Rhipicephalus in Africa, 4) colonization of medically important arthropods to meet research requirements.

Project Description:

This project pertains to the systematic treatment, vector relationships, biology, ecology, and colonization of medically important arthropods with the objective of elucidating the extra human dynamics of pathogens transmitted by such arthropods to man.

During this year a major review of the genus Ixodes of the United States has been completed. However, it will be necessary to publish this review on glossy paper, because of the 300+ SEM photos, and therefore a suitable publication medium has not been found.

A cooperative program from 1967 to present with the Oregon State Health Department has clarified the distribution of the 3 man-biting species in that state, Ixodes pacificus, Dermacentor andersoni, and D. occidentalis. This study involved collecting 11,839 ticks of the above 3 species in 35 of the 36 Oregon counties and revealed a slowly expanding range of the 2 indigenous Dermacentor species; a spotted fever group rickettsia was isolated from I. pacificus in an area of Oregon outside the known range of any spotted fever vector (see Project No. 00080-09). In addition, a possible new focus of D. variabilis, the American dog tick, was recorded.

In cooperation with Miss Jane Walker, Onderstepoort, who has reared many of the medically important rhipicephalids, we are undertaking a revision of certain medically important species groups of the genus Rhipicephalus found in Africa. The epidemiology of many of the livestock diseases found in Africa is clouded because the true taxonomic status of the tick vector is unknown. Scanning electron microscopic studies of all stages of reared material from numerous areas in southern and eastern Africa are beginning to clarify the species status of several rhipicephalid groups (i.e., pravus, punctatus, capensis, oculatus, etc.). The aim of this project is to provide accurate identification of the vector species involved without dependence upon SEM facilities.

Specimens of the extremely rare third family of ticks, Nuttalliellidae, were recently discovered in Tanzania. We have examined 14 of the 15 specimens still in existence and have redescribed Nuttalliella namaqua with the aid of SEM photos. A manuscript has just been completed on this monotypic family.

A large collection of parasitic mites of Surinam was identified, resulting in the recognition of 4 genera and species heretofore unknown. Manuscripts describing the new forms are in preparation. The taxonomically and biologically trained personnel of this section continue to provide numerous identifications for individuals, universities, research groups such

as USDA, etc., and are frequently consulted for information involving protection from and control of medically important arthropods.

The second and last year of a contract to computerize the tick data at RML and NAMRU-3 is nearing completion. From June 1974 through April 15, 1976, 61,706 RML cards and tick records were processed on Friden Flexowriter tape machines. A total of 95,400 records have been researched, but many cards did not contain information on ticks or were in some way not suitable for inclusion in the computer program. This phase of the contract is scheduled for completion on September 30, 1976.

The RML insectary operating at full capacity provided 115,546 tick specimens, representing 12 species in 7 genera, to RML scientists and outside collaborating research institutions; currently, 36 tick and 2 mite species are under colonization.

The PL-480 agreement entitled "Interrelationships between ticks, vertebrates (including humans), and infectious agents, especially viruses" between NAMRU-3 and NIH, DHEW, is entering its second year. On-site visits to NAMRU-3 were made in September 1975 by Drs. Keirans and Clifford where the future of this project was discussed with the Principal Investigator and Naval officials. At this time it appears that NAMRU-3 will terminate sponsorship of the agreement in September 1976 and the administrative responsibility for this project will be assumed by Cairo University. However, it is hoped that the Bibliography Staff will be allowed to occupy their present quarters on the NAMRU-3 grounds, which all concerned feel is necessary for retaining continuity in this work.

We plan to continue investigations into the systematics, vector relationships, disease transmission potential, and host interactions of medically important arthropods.

Publications:

Keirans, J. E.: A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). J. Med. Entomol. 12: 71-76, 1975.

Clifford, C. M., Hoogstraal, H., and Keirans, J. E.: The Ixodes ticks (Acarina: Ixodidae) of Nepal. J. Med. Entomol. 12: 115-137, 1975.

Brennan, J. M. and van Bronswijk, J.E.M.H.: Parasitic mites of Surinam. XXI. New records of Surinam and certain French Guiana chiggers with the description of a new species of Loomisia Brennan and Reed, 1972 (Acarina: Trombiculidae). J. Med. Entomol. 12: 243-249, 1975.

Publications: (cont'd)

Hoogstraal, H., Kaiser, M. N., Clifford, C. M., and Keirans, J. E.: The subgenus Persicargas (Ixodoidea: Argasidae: Argas). A. (P.) ricei, new species, a parasite of vultures in Texas. Ann. Entomol. Soc. Am. 68: 873-881, 1975.

Keirans, J. E.: The Rocky Mountain wood tick and American dog tick. In Great Plains Beef Cow-Calf Handbook. Garden City, Kansas, Great Plains States, Cooperative Extension Service, 1975, pp. 3252.1-3252.2.

Clifford, C. M., Theiler, G., and Baker, M.: Ixodes (Afrixodes) drakensbergensis n. sp. from domestic and wild animals in Natal, Republic of South Africa. Onderstepoort J. Vet. Res. 42: 33-40, 1975.

Keirans, J. E.: Records of phoretic attachment of Mallophaga (Phthiraptera: Insecta) on insects other than Hippoboscidae. J. Med. Entomol. 12: 476, 1975.

Reed, J. T. and Brennan, J. M.: The subfamily Leeuwenhoekinae in the Neotropics (Acarina: Trombiculidae). Brigham Young Univ. Sci. Bull., Biological Series 20: 1-42, 1975.

Brennan, J. M. and Reed, J. T.: A list of Venezuelan chiggers, particularly of small mammalian hosts (Acarina: Trombiculidae). Brigham Young Univ. Sci. Bull., Biological Series 20: 45-75, 1975.

Herrin, C. S. and Yunker, C. E.: Systematics of Neotropical Hirstionyssus mites with special emphasis on Venezuela (Acarina: Mesostigmata). Brigham Young Univ. Sci. Bull., Biological Series 20: 93-127, 1975.

Hoogstraal, H., Wassef, H. Y., Converse, J. D., Keirans, J. E., Clifford, C. M., and Feare, C. J.: Amblyomma loculosum (Ixodoidea: Ixodidae): identity, marine bird and human hosts, virus infection, and distribution in the southern oceans. Ann. Entomol. Soc. Am. 69: 3-14, 1976.

Poché, R. M. and Keirans, J. E.: Report of a tick, Ornithodoros rossi (Acarina: Argasidae), from the spotted bat, Euderma maculatum (Chiroptera: Vespertilionidae). J. Med. Entomol. 12: 503. 1975.

Keirans, J. E.: Dermacentor venustus Marx MS. in Neumann, 1897: proposed suppression under the Plenary Powers so as to conserve Dermacentor andersoni Stiles, 1908 (Acarina: Ixodidae). Z.N.(S.) 260. Bull. Zool. Nomencl. 32: 261-264, 1976.

In press:

Clifford, C. M., Flux, J.E.C., and Hoogstraal, H.: Seasonal and regional abundance of ticks (Ixodidae) on hares (Leporidae). J. Med. Entomol.

Stark, H. E., Inlao, I., Clifford, C. M., Keirans, J. E., Hoogstraal, H., and Lakshana, P.: Geographic distribution and hosts of ticks in Thailand. Appl. Sci. Res. Corp. J. Thailand.

Clifford, C. M., Walker, J. B., and Keirans, J. E.: Ixodes (Afrixodes) neitzi, n. sp. (Acarina: Ixodidae) from the mountain reedbuck in South Africa. Onderstepoort J. Vet. Res.

Clifford, C. M., Walker, J. B., and Keirans, J. E.: Ixodes (Afrixodes) bakeri Arthur & Clifford (Acarina: Ixodidae): description of the male and immature stages from rodents and insectivores in South Africa. Onderstepoort J. Vet. Res.

Clifford, C. M., Keirans, J. E., Hoogstraal, H., and Corwin, D.: Observations on the subgenus Argas (Ixodoidea: Argasidae: Argas). A. (A.) dalei, new species, parasitizing the Burrowing Owl in Peru. Ann. Entomol. Soc. Am.

Easton, E. R., Keirans, J. E., Gresbrink, R. A., and Clifford, C. M.: The distribution in Oregon of Ixodes pacificus Cooley & Kohls, Dermacentor andersoni Stiles, and Dermacentor variabilis (Say) (Acarina: Ixodidae). J. Med. Entomol.

Keirans, J. E., Clifford, C. M., and Corwin, D.: Ixodes sigelos, n. sp. (Acarina: Ixodidae), a parasite of rodents in Chile, with a method for preparing ticks for examination by scanning electron microscopy. Acarologia

Hoogstraal, H., Clifford, C. M., Keirans, J. E., Kaiser, M. N., and Evans, D. E.: The Ornithodoros (Alectorobius) capensis group (Acarina: Ixodoidea: Argasidae) of the Palearctic and Oriental region. O. (A.) maritimus: identity, marine bird hosts, virus infections, and distribution in western Europe and northwestern Africa. J. Parasitol.

Appendix 1. PL-480 Project 03-036-N. Interrelationships between Ticks, Vertebrates (including Humans) and Infectious Agents, especially Viruses, NAMRU-3, Cairo, Egypt.

Total Man Years: 15.0 Professional: 1.0 Other: 14.0

Annual Funding - $163,902

A major review of the previously obscure history and dynamics of Crimean-Congo hemorrhagic fever in Europe, Asia, and Africa is producing a solid background for understanding epidemiological factors and the risk presented by this disease. Several hundred papers translated from Russian (and also distributed to American colleagues) under the terms of this agreement have contributed much information for this review. Intensive, world-wide investigations of ticks and viruses of marine birds and of birds nesting in sheltered situations are leading to a significant revision of biological concepts in this area and to discovery of viruses causing human illness and mass bird die-offs. Basic studies on the identity of tick vector species are establishing or clarifying the role of individual species in virus epidemiology. The nearly completed first volume of the 2-volume Annotated Bibliography of Tickborne Viruses provides a valuable base for planning experimental and field research and assembles all available information for analysis for epidemiological principles and systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00070-11 RML

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biological Activities of Substances in Bordetella pertussis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. K. Bergman	Senior Scientist	RML NIAID
	J. J. Munoz	Research Microbiologist	RML NIAID
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION

Allergy and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the biological effects of substances from Bordetella pertussis with special emphasis on those substances which induce physiological and immunological responses in experimental animals. Areas of current investigation are: 1) the induction of hyperacute experimental allergic encephalomyelitis by pertussigen and its effect on vascular permeability in the rat's central nervous system utilizing radiolabeled human serum albumin, and 2) characterizing the differences between histamine hypersensitivities produced in mice by endotoxin and pertussigen.

Project Description:

The purpose of this project is to investigate in laboratory animals the biological effects of substances from Bordetella pertussis which produce physiological and immunological responses that are related to hypersensitivity and autoimmune phenomena.

Lewis rats were inoculated intraperitoneally with a purified extract of B. pertussis (pertussigen) and an aqueous emulsion of guinea pig spinal cord. At various intervals thereafter, the permeability of the vascular bed in the spinal cord and brain to radiolabeled human serum albumin (HSA) was determined. There was a striking increase, starting on the sixth day, in vascular permeability of rats that had received spinal cord antigen and pertussigen. Those that received cord alone showed only a slight increase in vascular permeability over normal rats. The onset of permeability changes corresponded with the development of paralysis. There was also some correlation of paralysis first occurring in the tail and hindquarters with elevated permeability in the lumbar-sacral region of the spinal cord. Pertussigen may produce hyperacute experimental allergic encephalitis (EAE) in Lewis rats through its ability to increase vascular permeability. This may occur from an enhanced reactivity of the vascular bed to vasoactive amines released at the site of antigen-antibody reactions; but not from direct damage to the nerve, since many rats recover completely from the paralysis of hyperacute EAE. The paralysis may result from a transitory impairment of nerve conductivity, possibly due to edema in the spinal cord.

Endotoxin from B. pertussis, Salmonella enteritidis, and Escherichia coli induced hypersensitivity to histamine in mice within 90 min after intravenous administration of 0.1 to 0.01 μg of endotoxin. This sensitivity was very transient and disappeared in 4 to 8 hr. Young mice (3 to 4 weeks old) were much less susceptible than were older mice (7 weeks old). Epinephrine given iv immediately after histamine challenge protected mice from death, but norepinephrine and isoproterenol were ineffective. The endotoxin activity could be precipitated with endotoxin antisera, but the activity was not neutralized, since the precipitate still had high histamine sensitizing activity. Endotoxin-induced histamine sensitivity apparently results from a short-lived impairment of the adrenergic regulation of the vascular bed, but this impairment seems different from that induced by pertussigen. The mechanism by which low doses of endotoxin produce the transitory histamine sensitivity is not known. Whether it acts by affecting a specific locus in the hypothalamus or perhaps by a more generalized toxicity on the microvasculature is not certain. The protection afforded by epinephrine would argue against death being caused by a hyperreactivity of the vascular bed to endogenously released epinephrine. Whatever the effect of endotoxin, it apparently does not produce any permanent damage and the mouse is able to overcome its effects within a few hours.

In future work we will investigate whether rats with hyperacute EAE have either humoral or cellular antibody to rat myelin basic protein and whether we can detect antibody on the myelin sheath of nerves in the spinal cord. The possible role of metabolites and other metabolic factors on the ability of pertussigen to produce histamine hypersensitivity and increased vascular permeability in mice will be investigated. The dysfunction of the adrenergic nervous system in pertussigen-treated mice will continue to be investigated. Catecholamine levels in mouse plasma following administration of endotoxin, pertussigen, histamine and other treatments will be measured by use of a very sensitive radioenzyme assay for epinephrine.

Publications:

Bergman, R. K. and Munoz, J. J.: Effect of Bordetella pertussis extract and vasoactive amines on vascular permeability. J. Allergy Clin. Immunol. 55: 378-385, 1975.

Bergman, R. K. and Munoz, J. J.: Vascular permeability in spinal cords and brains from rats with hyperacute EAE induced with the aid of pertussigen. IRCS 4: 202, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00071-06 RML

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Purification and Activities of Pertussigen - a Substance from Bordetella pertussis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Munoz	Research Microbiologist	RML NIAID
	R. K. Bergman	Senior Scientist	RML NIAID
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION

Allergy and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.7	1.2

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to characterize the substance from Bordetella pertussis cells which we have named "pertussigen". This substance is responsible for the induction of lymphocytosis and hypoglycemia, for increasing susceptibility to vasoactive amines and autoimmune diseases and for the stimulation of antibody production, especially of the IgE class. It also induces protection in mice against intracerebral challenge with B. pertussis. Highly purified pertussigen has been obtained, but in only small amounts. We have concentrated our efforts to improve yields so as to obtain enough pertussigen to fully characterize it chemically and biologically.

Project Description:

The main aim of this project is to characterize the substance from Bordetella pertussis cells which is responsible for most of the known biological activities of these cells. We have named this material "pertussigen" to avoid the present confusion created by different functional names, such as histamine sensitizing factor (HSF), lymphocytosis promoting factor (LPF), adjuvant factor and others. We have obtained preparations of pertussigen that are free of demonstrable contaminants. They contain mainly protein and lipid. Due to the low yields obtained, however, these studies have not been conclusive, and for this reason we have concentrated on improving the fractionation techniques to collect larger amounts. In a promising technique developed, we treated freshly grown cells with lysozyme, DNase and RNase, followed by precipitation at pH 4.5 and extraction of the precipitate with 1 M NaCl-0.05 M sodium pyrophosphate at pH 8.5. This extract was precipitated at pH 4.5, redissolved in 1 M NaCl Na-pyrophosphate and passed through either G-200 Sephadex or Biogel columns. This simplified procedure should allow us to collect enough pure material to study pertussigen in more detail.

Certain agents that mimic actions of pertussigen are under study. Infestation with some nematodes is known to increase the production of IgE. We found that Trichinella spiralis infection in mice renders them more susceptible to histamine. We have also found that T. spiralis infection makes mice more resistant to PCA reactions, not only performed with IgE but also to IgG_1 class of antibodies. This resistance to PCA begins at 15 to 20 days after infection and remains thereafter. The sera of T. spiralis infected mice have a substance that blocks PCA reactions. This substance is most likely IgE, since IgE myeloma protein from rats also blocked the PCA response induced with mouse IgE in either mice or rats. Rat IgE fixed to mouse mast cells and mouse IgE fixed to rat mast cells; hence, the 2 proteins must be closely related.

It is our hope that the full characterization of pertussigen will allow us to study its mechanisms of action more precisely and that this will lead to better understanding of the phenomena that it modifies, such as hemodynamic shock, autoimmunity, stimulation of IgE and other Ig classes, and the resistance of mice to infection with B. pertussis. Our studies with T. spiralis are opening a new field - the role of worm infestation on hypersensitivity reactions.

In the future we will study the various activities of purified pertussigen and will expand the studies with T. spiralis. We will attempt to find a substance in the worm that can increase the IgE production.

Publications:

In press:

Munoz, J. J. and Bergman, R. K.: Immunological and other biological activities of Bordetella pertussis. Marcel Dekker, Inc. (Monograph)

Appendix 1. PL-480 Project 05-042-N. Cytophilic Antibody and Transfer of Skin Reactions, Institute of Microbiology, Cracow, Poland.

Total Man Years: 3.7 Professional: 0.7 Other: 3.0

Annual Funding - $40,126

The macrophages from mice undergoing graft vs. host (GVH) reaction are nonspecifically cytotoxic to target cells of syngeneic, allogeneic or xenogeneic origin. It was shown that the cytotoxicity of macrophages depends on the stage of GVH reaction and their activity may be modified by antibodies to immunoglobulins. The activated macrophages seem to have alloantibodies on their surface and it has been postulated that this antibody is responsible for the activation. Preliminary experiments have shown that alloantibody of IgM type directed to the H-2 determinants increases the cytotoxicity of macrophages while those of IgG type do not, and even suppress macrophage activity.

In guinea pigs it has been possible to transfer delayed skin reactions by whole peritoneal exudate cells or by peritoneal macrophages without lymphocytes. These cells incubated with anti-IgG did not transfer skin sensitivity.

An alpha globulin can be isolated from different sources (human and bovine sera, mouse ascitic fluid, human ovarian cyst fluid) that when given to mice shortly before administration of oxazolone inhibited the contact sensitivity produced by this antigen. This alpha globulin, when given to F-1 hybrid mice, also inhibited the GVH reactions.

Publications:

Ptak, W. and Hanczakowska, M.: Alloantibody-induced cytotoxicity of macrophages. J. Immunol. 115: 796-799, 1975.

Ptak, W., Hanczakowska, M., and Skowron-Cendrzak, A.: Transplantation 19: 12-19, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00072-05 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Host Defense Mechanisms in Viral Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. L. Lodmell Senior Scientist RML NIAID
OTHER: None

COOPERATING UNITS (if any) Dr. A. L. Notkins, Laboratory of Oral Medicine, NIDR, NIH.

LAB/BRANCH
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SECTION
Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

SUMMARY OF WORK (200 words or less - underline keywords)

The major objectives of this project are to study immunological mechanisms of host defenses against acute, persistent, and latent viral infections. Systemic and local defenses are being investigated with reference to both specific and nonspecific immune mechanisms. In particular, the mutual interaction of antibody, lymphocytes, macrophages, and mediators are being examined to ascertain their various roles in the process of recovery from disease. Projects of present interest are: 1) the effect of tuberculin delayed-type hypersensitivity reactions on vaccinia virus infections in rabbit skin; 2) the effect of Mycobacteria and mycobacteria cell products on vaccinia virus infections in rabbit skin and systemic encephalomyocarditis virus and HSV-II infections of mice; and 3) the effect of previous virus infections on superinfection with an unrelated virus.

PHS-6040

Project Description:

The major objectives of this project are to study immunological mechanisms of host defenses against acute, persistent, and latent viral infections. These studies have focused on in vivo models for determining the effects of immunologically induced mediators, humoral immunity and cell-mediated immunity to vaccinia virus infections of rabbit skin and encephalomyocarditis (EMC) virus infections of mice.

The major emphasis the past year has been to determine the effect of tuberculin delayed-type hypersensitivity (DTH) reactions on vaccinia virus infections in rabbit skin. It was determined that tuberculin reactions initiated at sites of primary vaccinia virus infections inhibited virus replication by ≥90% at these sites, as compared to skin injected with phosphate-buffered saline or nonspecific irritants. It also was shown that high concentrations of early local acid stable nonimmune interferon at tuberculin reactions were associated with the dramatic early clinical and virological cure of these infections. In studies in uninfected skin, an immunologically induced acid labile interferon was detected at tuberculin DTH reactions.

In additional studies, it has been shown that whole cells or cell walls of Mycobacteria emulsified in oil protect mice from a lethal challenge of EMC virus. The vaccine is effective for at least 8 weeks after vaccination (last interval tested) and appears to enhance protection through specific and nonspecific mechanisms as approximately 50% of the survivors do not have serum neutralizing antibody to EMC. In additional studies with Mycobacteria cell products, it has been shown that an intradermal injection of 150 µg of BCG cell-wall-oil vaccine on one side of a normal unsensitized rabbit will completely inhibit the replication of vaccinia virus on the contralateral side.

These studies are significant because they provide additional insight into the current concept that specific humoral and cellular immune responses are intertwined with nonspecific components of immunity in host defenses against viral diseases. More specifically, this work has shown that the tuberculin delayed-type hypersensitivity reaction is beneficial in the host defense against dermal vaccinia virus infection. This result, in itself, is significant in that it has emphasized quite dramatically that DTH reactions can be additive in the host response to viral infection, rather than immunosuppressive as current work by other investigators would indicate. Furthermore, these studies provide a new method and idea for the treatment of persistent and latent viral infections as varicella zoster and herpes simplex.

These stimulating and provocative studies of immunological defense against varied viral diseases will continue to be pursued in the present and newly developed model systems.

Publications:

Notkins, A. L. and Lodmell, D. L.: Cellular immune response in viral infections. In Pollard, M. (Ed.): Perspectives in Virology IX. Antiviral Mechanisms. New York, Academic Press, 1975, pp. 115-134.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00073-11 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Immunity in Mammals and Lower Vertebrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Coe	Medical Officer	RML NIAID
OTHER:	B. W. Chesebro	Surgeon	RML NIAID
	J. L. Portis	Research Associate	RML NIAID
	M. E. Bloom	Research Associate	RML NIAID
	J. F. Bell	Medical Director	RML NIAID
	W. J. Hadlow	Research Veterinarian (Path.)	RML NIAID
	L. A. Thomas	Research Microbiologist	RML NIAID

COOPERATING UNITS (if any) Dr. Ira Green, NIAID, NIH, Bethesda, Md.; Dr. T. Kindt, Rockefeller University, N.Y.; Dr. U. Nilsson, University of Pennsylvania, Philadelphia.

LAB/BRANCH
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SECTION
Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	0.8	1.4

SUMMARY OF WORK (200 words or less - underline keywords)

The main goal of this project is to define immunological mechanisms in the Syrian hamster with particular emphasis on the role of various Ig classes. Experiments are devised to determine why only one Ig class may be preferentially stimulated depending upon antigenic presentation, what are the relative protective roles (phagocytosis promoting, tumor suppression or enhancement, etc.) of each Ig class and how can the immune response be channeled for synthesis in one or another Ig class.

Project Description:

The main goal of this project has been to define immunological mechanisms in the Syrian hamster. Further evidence for functional differences in hamster Ig classes has been obtained by testing hamster peritoneal mast cells for histamine release after reaction with rabbit antisera specific for various hamster Ig classes. Hamster peritoneal cells contain relatively large numbers of mast cells (approximately 15% of total) which release histamine after reaction with specific anti-γ_2 and γA but not with anti-γ_1 and γM, suggesting the presence of γ_2 and γA receptors on the mast cell surface. However, in contrast to studies with anti-γ_2, absorption of the anti-γA with homologous antigen (γA) still resulted in effective histamine release. This finding suggests the possibility that the anti-γA sera also contained antibodies to another protein bound to mast cell surfaces, i.e., γE.

We have found that lymphoid cells of hamsters may be characterized as T cells (thymus derived) or B cells (bone marrow derived) similar to other mammals. T cells, similar to thymocytes, characteristically lack surface membrane immunoglobulin and C_3 receptor but do contain a thymus antigen detectable by cytolytic assay with specific anti-thymus cell antiserum. B cells lack the thymus specific antigen(s) but do contain membrane bound Ig, and frequently also have a receptor for C_3. Ig class specific antisera were used to define by fluorescent microscopy the Ig classes on B cells. In normal splenic lymphocyte populations, cells with membrane bound IgM were detected most frequently (25%), and approximately 5% of cells contained γA and 7Sγ_2 whereas about 1% had 7Sγ_1 on their surface.

Although γ_2-B cells are a minority among the B cell population, 2 SV-40 induced lymphomas examined to date both contained only membrane bound γ_2, suggesting an association between γ_2-B cells and a SV-40 receptor. One of these lymphomas was found to be secreting a monoclonal 7Sγ_2 globulin detectable in serum of tumor bearing hamsters. Idiotypic specific antisera showed the monoclonal Ig in the cytoplasm and also in the membrane of the tumor cells. Lymphoid cells which secrete IgG and also have IgG in the membrane are a disputed entity in other B cell systems and future work will characterize the kinetics of the membrane bound Ig for secretor or membrane bound status. In contrast to that in normal hamster B cells, the tumor membrane bound Ig did not cap. Furthermore, rabbit anti-hamster Ig sera of various specificities did not kill the tumor cell by *in vivo* and *in vitro* assays, although antisera against the tumor cell (or antisera against normal hamster lymphocytes) are cytolytic. Ongoing experiments are aimed to define critical factors in hamster immunity (or enhancement which also has been observed) with these lymphoid tumors. Sera from male hamsters with tumors frequently contain a protein not detectable in normal males but present in normal adult females. Called Female Protein, its presence in males was induced by diethylstilbestrol and inhibited by testosterone.

Although the function of this protein is unknown, the hormonal dependence of hamster Female Protein is similar to that of Ss protein of mouse (recently shown to be the fourth component of complement) controlled by the Ss locus in the H_2 gene complex. Hamster Female Protein was found to have a sedimentation velocity of 7.3 and diffusion coefficient of 4.4 X 10^7, with a calculated molecular weight of 150,000. When analyzed in SDS-polyacrylamide gel electrophoresis (with or without a reducing agent), Female Protein had a molecular size of approximately 25,000 indicating that Female Protein is composed of 6 noncovalently linked subunits. Ongoing experiments are designed to elucidate the functional role of this protein and its possible relationship to the complement system.

Our laboratory also serves as a source of information and material for other scientists at the RML and University of Montana.

We plan 1) to explore immune mechanisms of hamsters against hamster lymphomas - in particular, the ability of 36-Ig (the monoclonal protein synthesized by and on the membrane of 36-lymphoid tumor), 2) to immunize and protect hamsters from 36 tumor challenge, 3) to characterize the Ig in the membrane of SV-40 lymphoid tumors (and other hamster lymphoid tumors) and compare it with Ig in the membrane of normal B cells, and 4) to elucidate the function and hormonal control of hamster Female Protein - particularly in relation to the complement system. Also, in collaboration with various investigators, we will continue our utilization of bullfrogs as a source of antibody with unique specificities to be used in various homology and "hidden determinant" studies.

Publications:

Portis, J. L. and Coe, J. E.: The immune response in the hamster. VII. Studies on cytophilic immunoglobulin. J. Immunol. 115: 693-700, 1975.

Portis, J. L. and Coe, J. E.: IgM the secretory immunoglobulin of reptiles and amphibians. Nature 258: 547-548, 1975.

In press:

Coe, J. E.: Ig synthesis by a SV-40 induced hamster lymphoma. Immunology

Coe, J. E., Leong, D., Portis, J. L., and Thomas, L. A.: Immune response in the garter snake (Thamnophis ordinoides). Immunology

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00074-04 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Host Defense Mechanisms in Chronic Viral and Neoplastic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. W. Chesebro	Surgeon	RML NIAID
OTHER:	J. L. Portis	Research Associate	RML NIAID
	D. L. Lodmell	Senior Scientist	RML NIAID
	J. E. Coe	Medical Director	RML NIAID

COOPERATING UNITS (if any) Dr. J. Stimpfling, McLaughlin Research Institute, Great Falls, MT; Dr. David Sachs, NCI, NIH, Bethesda, MD.

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SECTION
Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	0.9	1.3

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is the elucidation of genetically controlled host defense mechanisms involved in recovery from virus-induced leukemia in mice. Immune mechanisms influencing both control of virus infection and recovery from leukemia are being studied. Also, the antigenic characteristics of transplantable cell lines derived from virus-induced leukemias are being examined in terms of the relevance of these antigens to cell-cell interactions and recovery from leukemia.

Project Description:

The major goal of this project is the elucidation of genetically controlled host defense mechanisms involved in recovery from virus-induced leukemia in mice. A combined approach to this goal has been taken using techniques of genetics, immunology, and virology.

Friend mouse leukemia virus. In the Friend virus leukemia model a single gene (Rfv-1) which influences the ability of mice to recover from leukemia has been found to map in the D region of the major histocompatibility locus. Recovery from leukemia, mediated in part by the Rfv-1 gene, is also dependent on the presence of 3 to 5 other unidentified genes derived from the C57BL mouse genome. Presence of these 3-5 genes facilitates development of neutralizing anti-viral antibodies and cytotoxic anti-leukemia cell antibodies. These antibodies appear to be able to control and eliminate systemic viral infection, even in the face of ongoing progressive leukemia. In addition, mice that recover from leukemia produce splenic T lymphocytes which specifically recognize and kill leukemia cells in vitro, independently of macrophages or B lymphocytes. The Rfv-1 gene might possibly act by influencing the kinetics of production of these cytotoxic T lymphocytes; however, mice of all Rfv-1 genotypes tested have been observed to produce these cells under the appropriate conditions.

Future work in this system is aimed at further defining the role of T cells in recovery from leukemia by using neonatally thymectomized mice and passive transfer of immune and normal splenic T cell populations.

Properties of mouse leukemia cell lines. A number of mouse leukemia cell lines have been derived from primary leukemias induced by Friend and B/T-L (Tennant) leukemia viruses in vivo. These lines have been examined for the presence of infectious virus, viral antigens, and some normal cell antigens. Nine of 26 lines examined were positive for Ia antigens (antigens associated with I region of the H-2 complex), and 3 of these 9 Ia-positive lines also had a membrane receptor for complement. Such leukemia cells have not been described previously, and their origin is unclear at this time. Six of 26 lines bore the Thy-1 antigen. Ia and Thy-1 antigens were mutually exclusive. This suggested that these antigens may be markers of separate cell lineages.

The results described in both sections of this project have provided new information on mechanisms of action of humoral and cellular immune defenses in recovery from established leukemia in mice. The Friend virus leukemia model is a unique system in which it should be possible to characterize various distinct host defense mechanisms by means of genetic isolation of the individual genes which control each of these mechanisms.

It seems clear that detailed knowledge of all possible pathways of host immune defense against neoplastic disease is vital to understanding and manipulating of these defenses in the chronic viral and neoplastic diseases of man. Such detailed knowledge for human disease can best be acquired by the adaptation of data obtained from a large number of different laboratory virus and tumor model systems where experimental manipulation is unrestricted and genetic homogeneity can be obtained.

Future investigations are planned to determine the origin of the Ia-positive leukemia cells, and in collaboration with other groups, to characterize the biochemical nature of this antigen. In addition, the nature of the viruses and viral antigens expressed on these cell lines are under study to attempt to define the viral antigens involved in virus-specific cell-mediated immunity.

Publications:

Chesebro, B. and Wehrly, K.: Studies on the role of the host immune response in recovery from Friend virus leukemia. I. Antiviral and antileukemia cell antibodies. J. Exp. Med. 143: 73-84, 1976.

Chesebro, B. and Wehrly, K.: Studies on the role of the host immune response in recovery from Friend virus leukemia. II. Cell-mediated immunity. J. Exp. Med. 143: 85-99, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00075-02 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunology of Pathogenic Fungi

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. F. Hasenclever Scientist Director RML NIAID
OTHER: None

COOPERATING UNITS (if any)

None

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Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the immune response of laboratory animals to pathogenic fungi. The present emphasis is upon the humoral response as it may be affected by the schedule of immunization, the immunizing strain, the antigenic group or serotype, and sublethal infection.

Project Description:

The antigenic relationships among the serotypes of Saccharomyces telluris, the antigenic groups of Candida albicans and S. cerevisiae and the humoral response of laboratory animals to C. albicans or other yeasts are the major areas of study.

The antigenic differences among the serotypes of S. telluris can be shown in polysaccharides extracted from the yeast cells. Due to their cross reactions with antisera to C. albicans and S. cerevisiae, these polysaccharides are composed primarily of mannose. The major haptenic groups of the S. telluris Serotype A mannan, as determined by the similarity of its serologic reactivity with that of C. albicans Group A mannan, have been postulated.

Studies with several strains of C. albicans Group A have shown a variation in their immunogenicity. Some strains induce a higher and more consistent Group A specific antibody level in immunized rabbits than others. Rabbits react unpredictably to the less immunogenic strains in that some animals produce a good Group A specificity in their antisera but others fail to respond with specific antibodies.

Rabbits inoculated with sublethal doses of C. albicans may show no humoral response or detectable antibody levels, but never the immune stimulation elicited by vaccine immunized animals. At 2 months after inoculation, one could not predict whether chronic infection was established, and there was no correlation between chronic infection and presence of detectable antibody levels.

These studies are helping to characterize the humoral immune response to yeasts and yeast infections, permitting a more rational understanding of serology and the immune process in human disease.

Work on the above listed studies continues with new emphasis placed upon the immune response as influenced by polysaccharide antigens with slightly different molecular structures.

Publications:

In press:

Hasenclever, H. F. and Piggott, W. R.: Colonization of soil by Histoplasma capsulatum: II. Effect of bat guano added to an area of reduced colonization. Health Lab. Sci.

Hasenclever, H. F. and McAtee, F. J.: Antigenic relationships of Candida albicans, Saccharomyces telluris, and Saccharomyces cerevisiae. Proc. 21st Oholo Biological Conference on Host-Parasite Relationships in Systemic Mycoses, March 28-31, 1976, Ma'alot, Israel.

Hasenclever, H. F.: Impact of histoplasmosis: An airborne disease. Aerobiology Synthesis Volume, Aerobiology Program US/IBP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00076-18 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Immunopotentiation by Mycobacterial Fractions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. A. McLaughlin	Senior Staff Fellow	RML NIAID
	M. T. Kelly	Research Associate	RML NIAID
	E. Ribi	Research Chemist (Phys. Chem.)	RML NIAID
OTHER:	Y. Mizuno	Visiting Scientist	RML NIAID
	R. F. Smith	Research Chemist (Biochem.)	RML NIAID

COOPERATING UNITS (if any) Dr. R. Toubiana, CNRS Institute de Chimie des Substances Naturelles, Paris, France; Dr. R. Parker and S. M. Strain, Hamilton Biochemical Research Laboratory, Hamilton.

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SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	2.0	4.0

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to delineate at the cellular and molecular level the mechanism of action of microbial components in immunopotentiation. Four major approaches have been undertaken: 1) the determination of in vivo and in vitro activation of macrophages by fractions isolated from mycobacteria and rickettsiae, 2) measurement of chemotactic activity in vivo and in vitro of defined esters of trehalose isolated from a variety of mycobacteria, 3) chemical synthesis of analogs of these trehalose esters, and 4) characterization of the biological activity of these analogs.

Project Description:

In general, the major objective of this project is the definition of the structural requirements and mechanism of action of immunopotentiating components isolated from microbes. The capability of these components to cause regression of the line-10 hepatocellular carcinoma in guinea pigs is the criterion utilized to establish their activity as adjuvants or as immunopotentiating agents.

From the cell walls of Mycobacterium tuberculosis, strain Aoyama B and strain H37Ra, and from M. bovis have been isolated monomycolates of trehalose (P4) that have been shown to be potent adjuvants in protection against aerosol challenge to M. tuberculosis strain H37Rv and to cause regression of line-10 hepatocellular carcinomas. When combined with endotoxins from Re mutants of Salmonella typhimurium, these monomycolates are as effective in initiating events leading to tumor regression as are the previously described dimycolates of trehalose (P3).

To better understand the mechanisms of action of these microbial components at the molecular level, we conducted in vivo and in vitro studies in the search for a possible common element responsible for the granulomagenic and tumor regressive activity. By the proper combination of mineral oil with the mycolates of trehalose (in the absence of any antigenic substances), emulsions were prepared which produced tremendous lung and spleen weight increases in mice with but 10 µg dose of the mycolates. Utilizing an in vitro assay which measures the capability of a molecule to stimulate production of chemotactic factors from plasma, it was shown that only those molecules which possess in vivo granulomagenic activity are able to generate chemotactic substances from plasma. Thus, we propose that the mechanism of action of the trehalose mycolates in vivo involves in part the generation of chemotactic factors which attract cells to the tissues in which the oil droplets are localized.

With the use of structural analogs of these mycolic acid esters, including synthetic trehalose monomycolate, dimycolate, and diacetate, it has been possible to localize the active portion of the molecules. It appears that the active center in the generation of chemotactic substances is the trehalose moeity. However, trehalose alone is not active in producing granulomatous reactions in the lungs of mice or in generation of chemotactic factors from plasma. It appears that the trehalose must be esterified to a carbon chain although that chain need not be as long or as complex as that of the mycolic acids.

Correlative studies using the synthetic analogs in combination with endotoxins from the rough mutants of S. typhimurium indicate that not all analogs which are active in generation of chemotactic substances are active

as adjuvants in the tumor model system. For example, the diacetate of trehalose produced chemotactic substances but did not regress tumors when combined with endotoxin. Thus, it is clear that the trehalose moeity is an important component for the biological activity of P3 and P4, but it is also obvious that the fatty acid residues play essential, yet unknown, roles in the biological events culminating in tumor regression and in protection against infection with M. tuberculosis.

Several lines of evidence suggest that macrophages may play an important role in the mechanism of immunopotentiation by microbial agents. Therefore, we have investigated the effects of nonviable microbial immunostimulants on macrophage activation and chemotaxis. Two nonviable preparations, BCG cell walls and Q fever rickettsiae, induced activation of guinea pig macrophages in vivo and in vitro. Injection of the BCG cell wall vaccine or suspensions of killed C. burnetii into guinea pigs led to the accumulation of macrophages which were activated as measured by the ability to kill the intracellular pathogen, Listeria monocytogenes. Macrophage activation in vitro was measured by an assay for stimulation of macrophage glucosamine incorporation by microbial agents, and BCG cell walls and Q fever rickettsiae both induced 3-fold or greater stimulation. In addition, the BCG cell wall vaccine generated a chemotactic factor from plasma which led to the directed migration of macrophages. These studies suggest that modulation of macrophage function may be one important mechanism of immunopotentiation by microbial agents.

The future course of this project will include studies to correlate the structure and function of defined molecules which act as immunopotentiators. Studies are in progress to ascertain the nature of the interaction of the fatty acid esters of trehalose with endotoxin and of the endotoxin with the line-10 tumor cells. In addition, the effect of the trehalose esters in stimulating immune cell activity, such as phagocytosis and antibody production, will be studied. It is anticipated that the elucidating mechanism of action of chemically defined molecules will provide information useful in predicting feasible approaches to stimulation of the reticulo-endothelial system and cellular mediated immune response in man and other animals.

Publications:

Grangér, D. L., Yamamoto, K., and Ribi, E.: Delayed hypersensitivity and granulomatous response after immunization with protein antigens associated with a mycobacterial glycolipid and oil droplets. J. Immunol. 116: 482-488, 1976.

In press:

Ribi, E., Granger, D. L., Milner, K. C., Yamamoto, K., Strain, S. M., Parker, R., Smith, R. W., Brehmer, W., and Azuma, I.: Induction of resistance to tuberculosis in mice with defined components of mycobacteria and with some unrelated materials. J. Inf. Dis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00077-20 RML

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (30 characters or less)

Structure and Biologic Activity of Endotoxins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Milner	Scientist Director	RML NIAID
	E. Ribi	Research Chemist (Phys. Chem.)	RML NIAID
OTHER:	M. T. Kelly	Research Associate	RML NIAID
	C. A. McLaughlin	Senior Staff Fellow	RML NIAID

COOPERATING UNITS (if any) Dr. J. A. Rudbach and E. Milner, University of Montana, Missoula; Dr. R. Parker and S. M. Strain, Hamilton Biochemical Research Laboratory, Hamilton.

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SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.1	1.8

SUMMARY OF WORK (200 words or less - underline keywords)

This project has developed from the central purpose of elucidating the structural basis for host-reactive properties of bacterial endotoxins. Capabilities for physicochemical analysis and biological assay acquired in these studies have been applied to other projects of the RML Section of Molecular Biology. Currently, this application is entirely to studies of cancer immunotherapy.

Project Description:

Investigation of the physicochemical structure of endotoxin has been closely correlated, in this project, with special bioassays of endotoxic activity, because there is no chemical analysis for endotoxin, and only the biological activity can be quantitated. At present, the antitumor activity of particular endotoxins is being correlated with their other biological activities, such as pyrogenicity for rabbits, lethality for mice and chick embryos, specific and nonspecific protection tests in mice, and clotting of limulus amebocyte lysate. The mechanism of antitumor effect is also under study, and endotoxins are being chemically modified and fractionated in order to define the structures responsible for the activity. It is reemphasized that the effect currently studied is different from the well-known tumor-necrotizing effect of endotoxins. In our system, the endotoxin works synergistically with a nonsensitizing glycolipid from mycobacteria (P3 or P4) to produce permanent cures of established primary cancers and metastases to local lymph nodes.

Major findings include: 1) incorporation of endotoxins into immunotherapeutic agents for cancer, in our laboratory model, has greatly potentiated such agents; 2) the antitumor effect is negatively correlated with the immunogenicity of the O-antigenic component of endotoxin; 3) there is no correlation between antitumor effect and endotoxic potency; transmethylated "endotoxoids" were highly effective, as was an extract of low toxicity from Q fever rickettsiae; 4) two types of material from a given batch of dried, Re-mutant bacteria were about equally active: the cells could be extracted first with chloroform-methanol (4:1) then with hot phenol-water, and both extracts contained the activity; also, the order of extraction could be reversed, but the relative yields by each method remained about the same. The material obtained with chloroform-methanol was insoluble in water; the phenol-water extract was soluble in water but insoluble in chloroform-methanol.

The striking new findings of the potential role of endotoxins in immunotherapy of cancer have greatly increased the need for and productivity of this project. There is not only continued need for the bioassays but also renewed need for fractionation of endotoxins, because it appears that something less than a complete endotoxin - and perhaps some entirely nontoxic portion - may have the desired effect on malignant tumors. Although K. Milner reaches mandatory retirement 9 months after the present reporting period, well-qualified younger people, who would make highly desirable replacements, have expressed interest in joining this project. Dr. Rudbach, who has a much-needed fermentor, has been supplying us with material and is desirous of more extensive collaboration. He proposes to assign one of his graduate students (Eric Milner) to this project for prosecution of his Ph.D. research.

Publications:

Rudbach, J. A., Akiya, F. I., Elin, R. J., Hochstein, H. D., Luoma, M. K., Milner, E.C.B., Milner, K. C., and Thomas, K. R.: Preparation and properties of a national reference endotoxin. J. Clin. Microbiol. 3: 21-25, 1976.

Ribi, E. E., Granger, D. L., Milner, K. C., and Strain, S. M.: Brief communication: tumor regression caused by endotoxins and mycobacterial fractions. J. Natl. Cancer Inst. 55: 1253-1257, 1975.

In press:

Ribi, E., Takayama, K., Milner, K., Gray, G. R., Goren, M., Parker, R., McLaughlin, C., and Kelly, M.: Regression of tumors by an endotoxin combined with trehalose mycolates of differing structure. Cancer Immunol. Immunother.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00078-04 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Microbial Components in Cancer Immunotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Ribi	Research Chemist (Phys. Chem.)	RML NIAID
	C. A. McLaughlin	Senior Staff Fellow	RML NIAID
	M. T. Kelly	Research Associate	RML NIAID
OTHER:	K. C. Milner	Scientist Director	RML NIAID
	R. F. Smith	Research Chemist (Biochem.)	RML NIAID
	H. G. Stoenner	Vet. Officer Director	RML NIAID
	K. Yamamoto	Visiting Scientist	RML NIAID

COOPERATING UNITS (if any) B. Zbar and H. Rapp, NCI; W. Brehmer, R. Koch Inst., Berlin; J. Gutterman and S. Richman, M.D. Anderson Hospital, Houston; R. Parker and M. Strain, Hamilton Biochem. Res. Lab.; G. Gray, Univ. Minn.; M. Goren and P. Minden, Natl. Jewish Hosp., Denver; Y. Yamamura and I. Azuma, Osaka Univ.,Japan

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SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.9	3.1	5.8

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this project is to identify nonviable, chemically defined microbial components that are efficacious antitumor agents. The general approach in these studies is the development of standardized agents useful for both experimental and applied immunotherapy. The intent of this research is to 1) circumvent the use of infectious agents, 2) separate active components from potential antagonist substances, 3) remove or chemically modify components producing undesirable biological responses, and 4) develop and supply such efficacious preparations in stable and standardized forms.

Project Description:

Living Mycobacterium bovis, strain BCG, has been used for many years as an antituberculosis vaccine and has been found to be effective for treating certain neoplasms of man. Although BCG is relatively safe when used for vaccination against tuberculosis, frequent and sometime life-threatening complications, such as acute hypersensitivity reactions, have resulted from the use of viable BCG in cancer immunotherapy. Our finding that a nonviable BCG cell wall preparation was as effective as viable BCG in curing otherwise fatal tumors in guinea pigs demonstrated that the use of an infectious agent in cancer immunotherapy could be circumvented.

Doses of viable BCG that cure 50% of the line-10 tumors in guinea pigs produced large ulcerating lesions. However, very little reaction was produced by doses of BCG cell wall that brought about similar cure rates. Regression of these tumors was also achieved more rapidly with the cell wall preparation, as compared to viable BCG. The cell wall of BCG has also been used successfully to treat ocular squamous cell carcinoma of cattle. These highly invasive neoplasms provide a model more relevant to human neoplastic disease since the bovine ocular carcinoma is a spontaneous tumor occurring in an outbred population.

By fractionating the BCG cell wall, we have obtained chemically defined substances that retain antitumor activity. Such materials have the potential advantages of increased potency and reduced allergenicity. In our attempts to isolate and define the active components of the mycobacterial cell wall required for tumor regression, we first exhaustively extracted the cell walls with neutral organic solvents to liberate free lipids. These extracted cell walls were then digested with proteolytic enzymes to remove most of the protein moieties. The resulting cell wall skeleton possessed reduced antitumor activity. The activity was restored when the cell wall skeleton was combined with trehalose monomycolate or dimycolate which are also isolated from the cell wall. The mycolates of trehalose alone were not active.

Tests in human subjects conducted at the M.D. Anderson Hospital showed that administration of multiple intradermal doses of cell wall skeleton and trehalose mycolates produced some malaise, chills, and elevated temperatures of 101° F. However, the life-threatening complications such as "BCG-osis", anaphalactoid reactions, and contaminating bacterial supra-infections that may accompany treatment with viable BCG organisms have not occurred following administration of the cell wall skeleton plus trehalose mycolate. These nonviable microbial products have been administered to immunocompetent patients with stage I and stage II malignant melanoma by intralesional injection. Some of the treated nodules appear to be regressing.

Components from other microbes not related to mycobacteria also have antitumor activity. For example, aqueous phenol extracts of Coxiella burnetii (Q fever rickettsiae) in combination with trehalose caused complete regression of tumors in 60% of the treated animals. These extracts possessed a chemical composition typical for bacterial endotoxin, yet were several thousand times less toxic when compared to classical endotoxins. Saline suspensions of the whole organism, without oil or trehalose mycolates, regressed tumors in about 50% of the treated guinea pigs.

Lipopolysaccharides from mutant gram-negative bacteria, in combination with trehalose mycolates, were also found to be potent agents in bringing about tumor regression. The lipopolysaccharides alone occasionally regressed tumors whereas, when combined with the trehalose mycolates, the regression rate approached 100%. Earlier studies by others suggested that the microbial immunostimulants had to be injected directly into the tumors to be effective. However, we have found that intradermal injections made between the primary tumor and the draining lymph node, when followed by surgical excision of the primary tumor, resulted in elimination of lymph node metastases in 60% to 90% of the treated animals. Briefly, these experiments were conducted in the following manner. The combined microbial components or control placebo solutions were injected between the primary tumors and the first draining lymph node. Seven or 14 days following the injections, the primary tumors were removed surgically. All control animals were uniformly killed by metastatic tumor growth, whereas 90% of the animals treated with cell wall skeleton plus lipopolysaccharide plus trehalose mycolate survived, with no evidence of lymph node metastasis. These data may have considerable impact on the use of immunotherapy as an adjuvant to surgery in the treatment of spontaneous neoplasma of man and other animals..

Two major courses of action are planned for future research in this project. One is that of screening a wide variety of microbial components and combinations of these components for activity as immunopotentiators in bringing about tumor regression. The goal is to ascertain which are the most potent and generally useful components that produce minimal undesirable side effects. The second major objective is the isolation of the essential molecules which possess tumor regressive activity from those microbial components thus far studied.

Publications:

Gray, G. R., Ribi, E., Granger, D., Parker, R., Azuma, I., and Yamamoto, K.: Brief communication: immunotherapy of cancer: tumor suppression and regression by cell walls of Mycobacterium phlei attached to oil droplets. J. Natl. Cancer Inst. 55: 727-730, 1975.

Granger, D. L., Ribi, E., Azuma, I., Brehmer, W., Parker, R., Strain, S. M., and Smith, R. F.: Cancer immunotherapy with nonliving mycobacterial components. In Crispin, R. G. (Ed.): Neoplasm Immunity. Theory and Application. Chicago, Inst. for Tuberculosis Research, 1975, pp. 43-54.

Granger, D. L., Brehmer, W., Yamamoto, K., and Ribi, E.: Cutaneous granulomatous response to BCG cell walls with reference to cancer immunotherapy. Infect. Immun. 13: 543-553, 1976.

In press:

Ribi, E., Milner, K. C., Granger, D. L., Kelly, M. T., Yamamoto, K., Brehmer, W., Smith, R. F., and Strain, S. M.: Immunotherapy with nonviable microbial components. Ann. N.Y. Acad. Sci.

Zbar, B., Ribi, E., Granger, D., Evans, C., Rapp, H. J., and Kelly, M. T.: Immunological approaches to the treatment of human cancer based on a guinea pig model. Cancer Immunol. Immunother.

Kelly, M. T., Granger, D. L., Ribi, E., Milner, K. C., Strain, S. M., and Stoenner, H. G.: Tumor regression with Q-fever rickettsia and a mycobacterial glycolipid. Cancer Immunol. Immunother.

Ribi, E., Milner, K., Kelly, M. T., Granger, D., Yamamoto, K., McLaughlin, C. A., Brehmer, W., Strain, S. M., Smith, R. F., and Parker, R.: Structural requirements of microbial agents for immunotherapy of the guinea pig line 10 tumor. Proc. Int. Symp. on Present Status of BCG in Cancer Immunotherapy. Montreal, April 22-23, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00079-17 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Encephalitides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. A. Thomas	Research Microbiologist	RML NIAID
OTHER:	J. E. Coe	Medical Officer	RML NIAID

COOPERATING UNITS (if any) Dr. D. B. Lackman, Microbiology Laboratory, Montana State Dept. of Health, Helena; Dr. M. Skinner, Montana State Epidemiologist, Helena.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to determine the role garter snakes might play in the ecology of Western equine encephalitis. Of major importance are endeavors to demonstrate presence of antibody in garter snakes following experimental infection. Standard as well as modified serological techniques are employed. Assistance is provided upon request to various clinics, hospitals, and state health laboratories for isolation and identification of arboviruses and testing for hemagglutination-inhibiting and/or complement-fixing antibodies against these disease agents.

Project Description:

Western equine encephalitis (WEE) virus was active in the local region; both equine and human cases were detected. Of 116 suspect equine cases in Montana, 74 horses had hemagglutination-inhibiting (HI) antibodies, and 30 had complement-fixing (CF) antibodies; 14 had a 4-fold rise in HI antibody and 15 had a rise in CF antibody titer. Equine cases were also identified in Utah and Washington, and WEE virus was recovered from the brain of a horse in western Montana. One human case was confirmed in Montana. On the basis of a rise in antibody titer, 19 human cases of WEE and 7 human cases of St. Louis encephalitis were confirmed in North Dakota.

In view of reports by other investigators that "bedbugs" (Oeciacus vicarius), collected from swallow nests in eastern Colorado, were infected with WEE virus and may serve as an overwintering mechanism for this virus, Dr. Quickenden of Helena submitted approximately 200 bedbugs for testing and also about 400 fleas collected at a Wildlife Refuge near Malta, Montana, in January 1976. We failed to isolate any infectious agent from these collections.

The role of garter snakes in the ecology of WEE virus has been difficult to evaluate because antibody in these cold-blooded vertebrates after experimental infection with WEE virus could not be demonstrated with any degree of certainty. The past year we have developed a modification of our regular neutralization test, designated snake globulin precipitation (SGP) test, which we consider reliable for measuring antibody in garter snakes. Garter snakes were equally susceptible to WEE virus infection following either subcutaneous or intraperitoneal inoculation with WEE virus. Small doses of virus induced viremia in fewer snakes than did large doses. However, the magnitude of antibody development in viremic snakes was not dependent on the quantity of virus inoculated. With one exception, we did not detect viremia in snakes following a second inoculation of virus. It is not known how long neutralization antibodies persist in garter snakes. However, we detected these antibodies in high titer on the 823rd day after initial inoculation (snakes also received a second inoculation on day 74). These experiments are still in progress and attempts will continue to be made to identify the particular Ig class produced by snakes infected with WEE virus.

We will continue to perform diagnostic serologic tests for antibodies against selected bacterial, rickettsial, and viral diseases of interest to the Rocky Mountain Laboratory. The cooperative study of tick-borne viruses will be emphasized. The SGP test will be utilized in testing any sera collected from garter snakes from areas where WEE virus activity is evident.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00080-09 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tick-Borne Viral Disease Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. M. Clifford	Scientist Director	RML NIAID
	C. E. Yunker	Scientist Director	RML NIAID
OTHER:	J. E. Keirans	Research Entomologist (Medical)	RML NIAID
	R. N. Philip	Medical Director	RML NIAID
	L. A. Thomas	Research Microbiologist	RML NIAID

COOPERATING UNITS (if any) Dr. J. Casals, Yale Arbovirus Research Unit, New Haven, CT; Mr. R. Gresbrink, Oregon State Board of Health, Portland; Dr. H. Hoogstraal, NAMRU-3, Cairo, Egypt; Mr. Robin Rice, Dept. of Entomology, Univ. of Hawaii; Dr. J. E. George, Texas Tech University, Lubbock.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Medical Zoology and Zoonotic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.9	1.6	2.3

SUMMARY OF WORK (200 words or less - underline keywords)

Work on this project progresses along similar lines to that accomplished in 1974-75. Our collaboration with the Oregon State Health Department was continued and 3 strains of Colorado tick fever virus were recovered from Dermacentor andersoni. More significantly, 2 additional strains of Rocky Mountain spotted fever group rickettsia were isolated in Vero cells from Ixodes pacificus. Evidence of rickettsiæwas demonstrated in 7 pools of I. pacificus examined by immunofluorescent techniques. Numerous isolations of virus were made from Ornithodoros capensis during the past year. Specifically, 2 isolates of a virus closely related to Hughes virus were recovered from O. capensis collected on Aves Island in the Carribean; 63 strains of Soldado virus were recovered from O. capensis collected from bird islands off the coast of Texas; and of greater significance were 3 isolates recovered from these same pools of Texas ticks that are near but not identical to Upolo virus. Upolo was originally isolated from O. capensis ticks collected from the Great Barrier Reef near Australia. This Upolo-like agent has been characterized and is ready to be described and reported.

Project Description:

Attempts have been made to determine the incidence, distribution, and ecology of tick-borne diseases that influence man and domestic animals in the United States. Of equal importance is the investigation of the vector relationships of the increasing number of viruses known or thought to be associated with ticks that cause human illness.

Viruses in ixodid ticks. One hundred and sixty-four pools of hard ticks were examined for disease agents. Three strains of Colorado tick fever virus were recovered in suckling mice and Vero cells from 18 pools of Dermacentor andersoni from Malheur (1 strain) and Deschutes Co. (2 strains) in Oregon.

Rickettsia in ixodid ticks. Many of the 164 pools of hard ticks were also tested for the presence of rickettsia. Besides the inoculation of all ticks into Vero cells, many were also examined by Řeháček's hemocyte test and, more recently, they were tested by immunofluorescent procedures. Of 87 pools of Ixodes pacificus tested, one strain of R. rickettsii was recovered from Vero cells from a pool of 9 females collected in Lane Co., Oregon. Another pool of I. pacificus was positive in the hemocyte test and, more recently, 8 pools were considered positive for spotted fever organisms in the immunofluorescent tests, but isolations were made from only one. This isolate, recovered in Vero and Aedes albopictus cells, was derived from a pool containing 4 males and 4 females from Wasco Co., Oregon. Of the 18 pools of D. andersoni tested, 4 were determined to contain rickettsiae by Řeháček's hemocyte test, and a strain was isolated in Vero cells from a single female from Wasco Co., Oregon.

Viruses in argasid ticks. Two hundred and sixty-five pools of soft ticks were tested for the presence of viruses. Except for 26 pools from Tanzania, all pools tested were from species of ticks common to the Western Hemisphere.

Isolations were made from ticks from only 2 areas. Two isolates of a virus closely related to Hughes virus were recovered from 36 pools of Ornithodoros capensis collected on Aves Island in the Caribbean. Also, 63 strains of virus indistinguishable from Soldado were recovered from 164 pools of O. capensis collected at various islands adjacent to Texas. Also, 3 isolates of a new virus which is similar to, but not Upolo, were derived from these same ticks. This agent has been characterized physicochemically and serologically. It is an RNA virus 100-220 nm in size, has a lipid-containing outer envelope, and is sensitive to acid and heat. It crosses strongly in complement-fixing (CF), immunofluorescent (IF), and neutralization tests with Upolo virus, an ungrouped agent from related ticks in seabird colonies in Australia; however, these reactions are only a one-way

cross. Also, in contrast to Upolo virus, it is pathogenic for adult mice inoculated intraperitoneally. This virus will be described for publication and registered for inclusion in the World Catalog of Arboviruses.

A virus isolated from Tanzanian populations of Argas brumpti (see last year's report) has been tested exhaustively, with negative results, against our battery of tick-borne virus reagents. It has been sent to Dr. J. Casals (YARU) for additional testing.

Viruses from hosts of ticks. Two hundred and eleven blood samples from marine birds taken in the Pribiloff Islands in Alaska during Dr. Easterday's influenza studies were tested for arboviruses. A single isolate was recovered from the blood of a common murre. CF and IF tests on this virus indicate its relationship to Yaquina Head and Okhotskiy viruses (Kemerovo serogroup, Cape Wrath subgroup) from the USSR and Oregon, respectively. To our knowledge, no viruses of this subgroup have been recovered from vertebrates. Further study is planned.

Vector relationships. An adequate level of viremia caused by tick-borne viruses is necessary to effect transmission from reservoir host to vector. We continued to study induction of viremias by various North American tick-borne viruses. Four tick-borne agents of 3 serological groups were tested in suckling mice, newly hatched chicks, and domestic ducks by various routes of inoculation. Generally, viremias were brief and of low titer, but one virus, Tillamook, a member of the Sakhalin serogroup from Oregon, was recovered from duck blood in high titers for a prolonged period after intraperitoneal inoculation. This finding will facilitate transmission studies of this agent.

Work on this project will progress along similar lines to that accomplished last year. However, this effort is dependent upon a certain amount of field work to maintain its vitality and continuity. We have had to choose between meeting travel and field work and this has hampered some parts of our investigations. We have attempted to make up for the lack of travel by soliciting the aid of collectors in various parts of the United States. We have had some success with these collaborative efforts, but it would be preferable to have the mobility to follow up the leads that develop in this study with field trips of our own.

Publications:

Yunker, C. E.: Tickborne viruses associated with seabirds in North America and related islands. Med. Biol. 53: 302-311, 1975.

Yunker, C. E., Brennan, J. M., Hughes, L. E., Philip, C. B., Clifford, C. M., Peralta, P. H., and Vogel, J.: Isolation of viral and rickettsial agents from Panamanian Acarina. J. Med. Entomol. 12: 250-255, 1975.

Sonenshine, D. E., Yunker, C. E., Clifford, C. M., Clark, G. M., and Rudbach, J. A.: Contributions to the ecology of Colorado tick fever virus. 2. Population dynamics and host utilization of immature stages of the Rocky Mountain wood tick, Dermacentor andersoni. J. Med. Entomol. 12: 651-656, 1976.

In press:

Hughes, L. E., Clifford, C. M., Gresbrink, R., Thomas, L. A., and Keirans, J. E.: Isolation of a spotted fever group rickettsia from the Pacific Coast tick, Ixodes pacificus, in Oregon. Am. J. Trop. Med. Hyg.

Keirans, J. E., Yunker, C. E., Clifford, C. M., Thomas, L. A., Walton, G. A., and Kelly, T. C.: Isolation of a Soldado-like virus (Hughes group) from Ornithodoros maritimus ticks in Ireland. Experientia

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00081-15 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Epidemiology of Human Infections of Special Interest

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. N. Philip	Medical Director	RML NIAID
OTHER:	E. A. Casper	Nurse Director	RML NIAID
	L. A. Thomas	Research Microbiologist	RML NIAID
	R. L. Anacker	Research Microbiologist	RML NIAID
	W. Burgdorfer	Research Entomologist (Medical)	RML NIAID
	R. A. Ormsbee	Scientist Director	RML NIAID
	H. G. Stoenner	Vet. Officer Director	RML NIAID

COOPERATING UNITS (if any) Dr. J. N. MacCormack, North Carolina Dept. of Human Resources, Division of Health Services, Raleigh; Dr. C. M. Wilfert, Duke University Medical Center, Durham, NC.

LAB/BRANCH
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SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

This project emphasizes epidemiologic investigations of infectious diseases of particular interest to the research program of the Rocky Mountain Laboratory. Current investigations include a study of Colorado tick fever in the Northwest and the evaluation of improved methods for serodiagnosis of rickettsial infections, particularly Rocky Mountain spotted fever.

Project Description:

1. Colorado tick fever (CTF). Studies continue on the epidemiology of this disease in the Northwest. This year, 60 cases were identified by virus isolation from 174 blood samples submitted to RML by practicing physicians in Montana, Wyoming, and Idaho. Rapid diagnosis (on the day specimens were received) was possible in 73% of cases by fluorescent antibody staining of CTF antigen within erythrocytes.

Twenty-three cases occurred in Ravalli County. Several patients acquired disease on the east side of the Bitterroot Valley. Transmission of CTF virus by blood transfusion was demonstrated for the first time. A Hamilton resident donated blood to the American Red Cross 18 hours before showing signs of illness. Seven days later, the unit of blood was transfused into a Miles City, Montana cancer patient who subsequently had a prolonged, febrile, but ill-defined illness. CTF virus was isolated from the blood of the donor, the tubing used to obtain the contaminated unit, and from the blood of the recipient. Both the donor and the recipient also developed antibodies to CTF virus.

Over 4,000 sera from Red Cross blood donors from all parts of Montana were screened for CTF antibodies by immunofluorescence. Only 37 were reactive; evidently CTF did not occur often in this population group.

One thousand adult Dermacentor andersoni, collected from various west and east-side canyons, were examined by a hemolymph test for CTF antigen by direct immunofluorescence. The overall prevalence of CTF-positive ticks was 6.6%. Ticks taken from 82 persons were also examined by this test. Five were positive and the presence of CTF virus in 3 was confirmed by mouse inoculation (the other 2 were not tested in mice). However, only one of the 3 persons hosting definitely infected ticks later developed CTF. The usefulness of this procedure will be evaluated further during the coming tick season.

2. Rickettsial infections. The sero-epidemiologic study of Rocky Mountain spotted fever (RMsf) in collaboration with the North Carolina Division of Health Services (NCDHS) has been completed and the information is being assembled for publication. Comparison of serologic findings by various tests with clinical information indicated that the complement fixation (CF) test used at NCDHS, as well as that used for many years at RML, identified only 50% of the RMsf cases that actually occurred in the study group. There was good agreement among the more sensitive micro-agglutination, hemagglutination and microimmunofluorescence (MIF) tests. MIF tests using conjugates specific for a particular immunoglobulin class indicated that antibodies to R. rickettsii in CF-nonreacting sera were predominantly IgM rather than IgG.

In collaboration with Duke investigators, rickettsiae were isolated by Dr. Burgdorfer from 6 North Carolina RMsf patients. These strains were compared to 5 recent isolates from Montana RMsf cases in MIF tests of mouse antisera and convalescent patient sera. The eastern and western isolates were indistinguishable, and all were identical to the Sheila Smith strain used as immunogen in RMsf vaccine.

Although all strains recovered from RMsf patients thus far appear to be R. rickettsii, other tick-borne rickettsiae of the spotted fever group indigenous to this country should also be considered as possible human pathogens. We will attempt to explore this possibility during the coming year.

Publications:

Philip, R. N., Casper, E. A., Cory, J., and Whitlock, J.: The potential for transmission of arboviruses by blood transfusion with particular reference to Colorado tick fever. In Greenwalt, T. J. and Jamieson, G. A. (Eds.): Transmissible Disease & Blood Transfusion. New York, Grune & Stratton, Inc., 1975, pp. 175-196.

Philip, R. N., Casper, E. A., and Randall, W. H.: Transmission of Colorado tick fever virus by blood transfusion - Montana. CDC Morbidity and Mortality Weekly Report 24: 422 & 427, 1975.

Philip, R. N., Casper, E. A., Ormsbee, R. A., Peacock, M. G., and Burgdorfer, W.: Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. J. Clin. Microbiol. 3: 51-61, 1976.

South, P. J. and Stoenner, H. G.: The control of outbreaks of leptospirosis in beef cattle by simultaneous vaccination and treatment with dihydrostreptomycin. In Proceedings 78th Annual Meeting of the U.S. Animal Health Association, Roanoke, Virginia, October 13-18, 1974. Richmond, Va., U.S. Animal Health Association, 1975, pp. 126-130.

Stoenner, H. G.: Treatment and control of leptospirosis. In Johnson, R. C. (Ed.): The Biology of Parasitic Spirochetes New York, Academic Press, 1976, pp. 375-388.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00082-15 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

PROJECT (30 characters or less)
Relation of Viruses to the Genesis of Chronic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

:	W. J. Hadlow	Research Veterinarian (Path.)	RML NIAID
OTHER:	J. E. Coe	Medical Officer	RML NIAID
	R. E. Race	Veterinary Officer	RML NIAID

COOPERATING UNITS (if any) Dr. C. M. Eklund, Hamilton, MT (consultant); Dr. A. L. Klingsporn, USDA Animal and Plant Inspection Service, Hyattsville, MD; Dr. S. B. Prusiner, Dept. of Neurology, University of California, San Francisco.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Slow Viral Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13.6	2.2	11.4

SUMMARY OF WORK (200 words or less - underline keywords)

The abiding concern of this project is to characterize several naturally occurring slow viral diseases of domestic animals that resemble important, etiologically obscure diseases of man. Clinicopathologic, virologic, and immunologic features of the animal diseases are studied to obtain an understanding of the natural history and pathogenesis of scrapie and progressive pneumonia of sheep and Aleutian disease and transmissible encephalopathy of mink. Information obtained from such studies should provide not only an understanding of the mechanisms by which viruses cause protracted disease but also investigational techniques that may help to determine whether viruses are causally related to certain subacute or chronic diseases of man.

Project Description:

The project is concerned with 4 naturally occurring slow viral diseases of domestic animals: scrapie and progressive pneumonia of sheep and Aleutian disease and transmissible encephalopathy of mink. With each, the main objective of the research is to study the disease process, though some effort is also made to obtain information on the chemical and physical properties of the causal virus. Knowledge of the temporal distribution of virus, appearance of antibody, and host cellular responses and their relation to onset of disease is considered necessary before any concept of pathogenesis can be formed. These features, therefore, continue to receive most attention.

1. Scrapie and mink encephalopathy. Virologic information obtained from a study of Suffolk sheep naturally infected with scrapie virus provides a reasonably clear picture of the pattern of virus replication in this host. Major replication occurs in the intestinal tract, lymphatic tissues, and central nervous system (CNS). Thus, virus was detected in suprapharyngeal lymph node, tonsil, mesenteric-portal lymph nodes, ileum, spleen, and peripheral lymph nodes in 8 of 14 clinically normal yearling sheep from families with a high prevalence of the disease. Early appearance of virus in suprapharyngeal lymph node and tonsil suggests that infection occurs via the oral route with subsequent spread of virus to lymphatic tissues generally. Similar widespread distribution of virus was found in one of three 2-year-old clinically normal sheep. This pattern of non-neural infection was observed also in 10 Suffolk sheep during the clinical phase of the infection.

Virus was first detected in the CNS in the 2-year-old normal sheep, which had limited microscopic changes of scrapie in the thalamus. Because the largest amount of virus was found in the medulla oblongata in this sheep, it may be the site where virus enters the CNS. In ten 3- to 4-year-old sheep affected with the disease, concentrations of virus in the CNS were greatest in midbrain, diencephalon, and medulla oblongata early in the clinical course. Later, the cerebellar cortex contained the greatest amount.

Of particular concern in understanding the mode of infection with scrapie virus has been our failure to detect virus in 8 normal lambs less than 10 months old obtained from the same flock. This has yet to be explained. It is tentatively viewed as an extremely long eclipse phase, for other virologic findings suggest that infection occurs after birth by contact with an infected ewe.

To help clarify the question of in utero infection and vertical transmission, sheep fetuses (Suffolk) inoculated intramuscularly (neck) with scrapie virus (sheep brain origin) at about 100 days gestation were killed at intervals up to 322 days later and examined for virus. (Gestation period of sheep is 150 days.) To date, extremely small amounts were detected in lymph nodes regional to the site of inoculation (suprapharyngeal and prescapular) in only 2 lambs killed 255 and 300 days after inoculation. It is too early to draw conclusions from this finding, and much of the work needs to be repeated.

Further evaluation of virologic findings in mink inoculated with the Idaho isolate of encephalopathy virus suggests that natural occurrence of the disease represented accidental infection with what is now believed to be scrapie virus. After subcutaneous inoculation, encephalopathy virus replicated initially in the regional lymph node and then somewhat later in other peripheral lymph nodes but in no other tissues until it appeared in the CNS at about 20 weeks. Concentrations of virus were always low. Once infection in the CNS became well-established, virus appeared in many other tissues, such as spleen, liver, kidney, salivary glands, and intestine. This pattern of temporal distribution of virus suggests that the Idaho isolate of encephalopathy virus has only a limited capacity to replicate in non-neural tissues of mink and that infection of the CNS increases the susceptibility of these tissues to infection with this virus. The temporal distribution of virus in mink inoculated intracerebrally with scrapie virus of sheep origin has not been similarly studied.

Occurrence of scrapie in sheep and goats inoculated intracerebrally with mink encephalopathy virus (Idaho isolate) was reported previously. Since then, 4 of 5 goats inoculated with first passage goat brain became affected with the disease after an incubation period of 12 to 14 months. This contrasts with the 30 to 36 months observed in the first passage. Damage to the cerebral cortex was especially pronounced in the second passage goats. This is the only neuropathologic feature that distinguishes the experimental disease from typical scrapie. Such cortical damage is characteristic of encephalopathy in mink. One passage of this virus in goats did not render it pathogenic for mice. Second passage goat brain will be similarly examined.

2. Aleutian disease (AD). Because of disappointing results in producing infection in sapphire mink by oral inoculation of AD virus, intranasal inoculation was selected as an alternate peripheral route to simulate one possible natural mode of infection. As judged by either the occurrence of overt disease or the production of antibody alone, the intranasal route proved extremely effective with each of 3 strains of virus used. The pattern of morbidity and appearance of antibody in sapphires and

pastels were much like those usually observed in these genotypes after intraperitoneal inoculation with the particular strain of virus. Titration of virus intranasally usually gave endpoints (LD_{50}) about one $\log_{10}$ less than by intraperitoneal inoculation; they were about the same as those after subcutaneous inoculation. Variations in virulence of the different strains of virus were still evident in results obtained in pastel mink inoculated intranasally. These findings now provide an experimental system for closer examination of virus replication in mink exposed by a route that may explain the mode of horizontal spread of virus under natural conditions.

During the past year, the counter immunoelectrophoresis (CIEP) test for AD virus antibody has been used widely on mink ranches, especially in the Midwest. Great disparity has been observed between results of this test and those of the iodine agglutination test (IAT) commonly used in the field to detect AD by measuring elevated serum gamma globulin. This has caused great concern about the practical use of the CIEP test under field conditions and has brought to light questions about the epidemiology of AD that hitherto have received little attention. Our results on the variation in virulence of AD virus strains, persistence of virus in pastels (both reported previously), and the extensive use of CIEP test in the laboratory helped clarify much of the problem. For example, we found the CIEP test to be a highly specific, sensitive test for AD virus antibody that results from either present or past infection, in the latter subclinical. It does not detect disease as tests for elevated serum gamma globulin do. Therefore, the significance of results obtained with the CIEP test vis-a-vis those obtained with IAT must be evaluated in the light of several variables that may exist in a given circumstance. These are: predominant genotype of mink (Aleutian or non-Aleutian) in the total population, strain of virus causing the infection, and stage of the infection in individual mink or the herd when the test is done. Antibody resulting from subclinical infection persists in low titer for several years.

3. Progressive pneumonia. Remaining goats inoculated intravenously with progressive pneumonia virus were killed up to 3 years later and examined for virus. None was detected in lung, choroid plexus, mediastinal lymph node, and spleen. Studies on this disease were then discontinued.

Studies done so far in this project indicate that these four slow viral diseases present unusual host responses, all of which are extremely poorly understood. A better understanding of them should aid in the understanding and, hence control, of some important diseases of man.

Despite the many years this project has been devoted to the problem, much remains to be done to improve our understanding of these slow diseases. Although the kind of approach used to date can still be pursued profitably,

it should now be complemented by more sophisticated studies at the bench. Much of the necessary ground work has been provided, but persons with interest in the problem and with competence to carry out critical experiments are not at hand. Moreover, after nearly 15 years of full time work in the project, the current principal investigator wishes to be relieved of major responsibility for it. Thus, unless persons interested in continuing studies that emphasize the disease process are found and added to the project, preferably on a full time basis, experiments now in progress will be concluded over the next 3 years and the project, at least in its present format, will then be discontinued.

Publications:

Eklund, C. M. and Hadlow, W. J.: Slow viral and related diseases. In Hubbert, W. T., McCulloch, W. F., and Schnurrenberger, P. R. (Eds.): Diseases Transmitted from Animals to Man (ed. 6). Springfield, Ill., Charles C Thomas, 1975, pp. 1018-1028.

Bloom, M. E., Race, R. E., Hadlow, W. J., and Chesebro, B.: Aleutian disease of mink: the antibody response of sapphire and pastel mink to Aleutian disease virus. J. Immunol. 115: 1034-1037, 1975.

Bloom, M. E.: Aleutian disease of mink: production of ^{14}C-labeled antiviral antibodies by mink lymphoid cells in vitro. Infect. Immun. 13: 281-283, 1976.

Bloom, M. E. and Jackson, T. A.: Virus-like particles in buffy coat cells of normal goats and goats infected with progressive pneumonia virus. Am. J. Vet. Res. 36: 789-793, 1975.

In press:

Eklund, C. M. and Hadlow, W. J.: Animal viral diseases as models for central nervous system disease in man. In Vinken, P. J. and Bruyn, G. W. (Eds.): Handbook of Clinical Neurology. Amsterdam, North Holland Publishing Co.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00083-08 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Host-Ectoparasite Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. F. Bell Medical Director RML NIAID
OTHER: None

COOPERATING UNITS (if any) Dr. W. A. Nelson, Canada Dept. of Agriculture, Lethbridge.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Medical Zoology and Zoonotic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives are to discover the mechanism of acquired resistance to lice (in mice) and to distinguish that kind of resistance from native resistance and from the systemic kind of acquired resistance that occurs in tick infestation. Recent emphasis in our collaborative studies has been on histology and cytology at the several stages of reactivity to lice and to ticks and the effects of cortisone and of cyclophosphamide on cellular reaction. Differences in reactions between resistant and susceptible strains of mice are incorporated into the study program.

PHS-6040

Project Description:

Attacks on mammals by ectoparasites are characterized by reactions of immunoallergic nature. Repeated attacks usually result in acquisition of resistance with or without associated epidermal reactions. Frequent association of the reactions with development of resistance has often led to presumption of relationship, but we have evidence that the mechanism of resistance is much more subtle. However, acquired resistance to ticks is systemic while resistance to lice is localized to the site of previous exposure. Therefore, there must be at least 2 kinds of acquired resistance. We are studying the cytology-histopathology of both types in an attempt to define the mechanism that precludes feeding on the animal with acquired anergic resistance. Both cortisone and cyclophosphamide ablated acquired resistance to lice in mice and the effects of those substances on histologic changes of the resistant skin area will, we hope, provide a clue. All specimens necessary for study have been obtained and sections are being prepared, but microscopic examination has not been completed. A reaction of tolerance to lice occurred in some mice and skins of those animals are being examined to attempt to discern differences in histology. Attempts to produce the tolerant state by neonatal exposure have failed.

A review of Host-Ectoparasite Relationships, Part II, has been completed.

Publications:

Nelson, W. A., Keirans, J. E., Bell, J. F., and Clifford, C. M.: Host-ectoparasite relationships. J. Med. Entomol. 12: 143-166, 1975.

In press:

Stewart, S. J., Bell, J. F., Hestekin, B., and Moore, G. J.: Polyplax serrata: effects of limb disability on lousiness in mice. VI. Lack of tolerance after neonatal exposure. Exp. Parasitol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00084-07 RML

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Vector-Pathogen Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. E. Yunker	Scientist Director	RML NIAID
OTHER:	C. M. Clifford	Scientist Director	RML NIAID
	J. E. Keirans	Research Entomologist (Medical)	RML NIAID
	L. A. Thomas	Research Microbiologist	RML NIAID
	U.K.M. Bhat	NIH Visiting Fellow	RML NIAID

COOPERATING UNITS (if any) Dr. Kouka S.E. Abdel-Wahab, Al Azhar University, Cairo, A.R.E.; Dr. H. Hoogstraal, NAMRU-3, Cairo, A.R.E.

LAB/BRANCH
Rocky Mountain Laboratory, Hamilton, MT 59840

SECTION
Medical Zoology and Zoonotic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This program is designed to investigate the host-range, cellular relationships and growth dynamics of vector-borne pathogenic microorganisms (particularly arboviruses) in arthropods or their cells. Efforts are made to develop systems of arthropod (acarine and insect) tissue and cell culture and to apply them to the study of vector-borne disease agents. Arboviruses and rickettsias are tested for ability to propagate in these cultures and the ranges of pathogen supporting ability of the cell systems are thus defined. After prolonged growth in vitro and at the relatively low temperatures required by insect cells, pathogens are tested for changes in strain, virulence or antigenic characteristics. By contract, with a collaborating laboratory overseas, critical factors influencing the survival of tick vectors of disease agents and of these agents within the vectors themselves are being investigated from a biochemical and physiological standpoint. This last portion of the project also provides basic descriptive information necessary for progress in developmental tick tissue culture studies.

Project Description:

The objective of this project is to investigate the host-range, cellular relationships, and growth dynamics in bloodsucking arthropods or their cells of pathogenic agents transmissible by vectors among man or animals.

Studies were continued on the latent syncytial agent (ALSA3) that we isolated and described earlier from Singh's Aedes albopictus cell line that had been maintained by another laboratory. The agent is apparently a Togavirus, closely related serologically to Chikungunya virus, but differing in certain respects. This year we found that ALSA3, over many passages in carrier cultures of mosquito cells, retained its ability to cause syncytia formation and plaques in an uninfected strain of Ae. albopictus cells but lost this ability upon a single passage in Vero cells. "Cured" Ae. albopictus cells that had been previously infected with ALSA3 produced identical amounts of ALSA3 when reinfected as when first infected, but the former failed to show syncytia. Because of the close relationship of ALSA3 to Chikungunya virus, we studied the latter in mosquito cells. As previously noted, virulence of this virus for mice can be modified by growth in mosquito cells. However, our tests indicate that culture conditions of the mosquito cells may influence virulence of the virus and that partial reversion to virulence over a long term is possible. We will continue to define the parameters of these virus/vector- and virus/host-cell relationships, with special emphasis on pH of media and temperature of incubation. Results may provide an understanding of the influence of the vector cell on infectivity and pathogenicity of viruses. The demonstration that virulence of certain arboviruses may be manipulated by growth in arthropod cell cultures may hold significance for vaccine development.

Cell lines established from vectors of arboviruses have proven useful in the isolation, identification, and classification of these pathogens. This year we noted that the line of cells established by Peleg from Ae. aegypti mosquitoes would sometimes respond to infection with dengue-2 virus by forming syncytia. These inconsistent results prompted us to clone this cell line, whereupon it was found that 3 of 10 clonal populations consistently responded with more syncytia and higher immunofluorescent (IF) titers for dengue-2 virus than the uncloned population. Two of these clones equalled or surpassed Singh's Ae. albopictus cells in these respects. These results tend to confirm previous observations that only certain cells of a population are involved in virus production. They also indicate a potential utility of Peleg's cells in the study of dengue-2 virus. (Dengue viruses are considered among the more difficult arboviruses to isolate and assay.) However, these cells fail to plaque dengue-2 virus as efficiently as those of Ae. albopictus. This year additional tests of virus-plaquing ability of mosquito cells involved 3 additional lines of Aedes cells,

including 2 lines developed by us, and 2 of Culex cells. None of these lines proved equal to the Ae. albopictus line of Singh, which we studied earlier.

Applications of tissue culture methodology to the study of rickettsial organisms were continued. Previously we reported isolations in insect cell cultures of a Wolbachia-related agent from wild-caught chipmunks. These organisms could not be isolated in embryonated eggs, guinea pigs, or voles. This year, 6 additional isolations of this agent from the same species of sciurid were made in cell culture. None of 23 flying squirrels or 13 red squirrels trapped in the same area harbored the organism, but one of the flying squirrels, as well as 2 snowshoe hares, yielded Rocky Mountain spotted fever rickettsiae in cell cultures. The Wolbachia-like agent was studied by the indirect micro-IF test, as used by Dr. Philip of this laboratory. Results, which indicate its relationship to Wolbachia persica and W. andersoni but not to other rickettsiae or rickettsialike organisms, confirm previously obtained complement fixation findings.

Major progress in developmental arthropod tissue culture was made with the establishment of tick cells in culture, apparently as a serially propagating-permanent cell line. The species involved is the rabbit tick, Dermacentor parumapertus, and cells are now in their 21st passage, 11 months after primary explantation. This rewarding accomplishment came after 12 years of efforts in which, at best, tick cells could be maintained in good condition for long periods of time, but deteriorated upon subcultivation. Previously, cells from only one other species of tick, Rhipicephalus appendiculatus, had been established in culture. Karyological studies of the D. parumapertus cells indicate that the line is 85% diploid, with fibroblast-like cells predominating. Multiplication of viruses in the line is currently under study. Preliminary results indicate that the cell line, like primary tick tissue cultures studied previously, shows less ability to differentiate biologically between tick- and mosquito-borne viruses than do mosquito cells. Additional characterizations of the D. parumapertus cell line will be made. Also, despite our failure to understand the reason for successful adaptation of the D. parumapertus cells to growth in vitro, we have renewed attempts to establish a line of cells from the medically important species, D. andersoni. During the past year, 77 cultures of cells of the latter species were made, and from these approximately 260 subcultures were obtained. None survived beyond the 9th passage level.

We continued to supply, upon request, arthropod cells, viral stocks, antisera and tissue culture antigens to various federal, state and university laboratories, including NIH, and to train visiting researchers in arthropod tissue culture techniques.

Publications:

Cory, J., Yunker, C. E., Howarth, J. A., Hokama, Y., Hughes, L. E., Thomas, L. A., and Clifford, C. M.: Isolation of spotted fever group and Wolbachia-like agents from field-collected materials by means of plaque formation in mammalian and mosquito cells. Acta Virol. 19: 443-445, 1975.

Hirumi, H., Hirumi, K., Speyer, G., Yunker, C. E., Thomas, L. A., Cory, J., and Sweet, B. H.: Viral contamination of a mosquito cell line, Aedes albopictus, associated with syncytium formation. In Vitro 12: 83-97, 1976.

Appendix 1. PL-480 Project 03-016-1. Biochemistry and Physiology of Tick Vectors, NAMRU-3, Cairo, Egypt.

Total Man Years: 3.5 Professional: 1.0 Other: 2.5

Annual Funding - $4,716

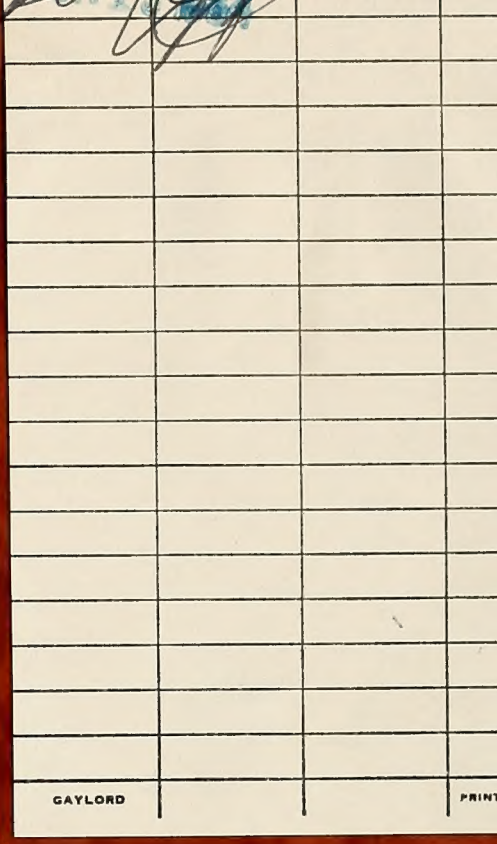
This productive project has yielded over 50 research articles and resulted in 5 Ph.D. and 4 M.Sc. theses. It was terminated September 30, 1975, and is proposed for reactivation at the National Research Council of Egypt. Published findings to date are in the general areas of water relationships, oxygen consumption, and inorganic and biochemical composition of biological fluids. Specific subjects are: new biochemical techniques; standard physiological states for analyses; salivary gland histology; digestive systems; blood-meal hemolysis; excretory system and nitrogenous excretory products; spiracular morphology and function; gonad development, oogenesis, oviposition; morphogenesis; juvenile hormone termination of diapause; hemolymph and egg pigment; cuticular permeability and water balance; osmotic pressure and ionic regulation; oxygen consumption; fatty acid, phospholipid, sterol, free amino acid, protein and sugar content of cell-free biological fluids; a new fatty acid; hemocyte characterization and hemolymph volume; life cycles, ecology and seasonal dynamics. Additional papers on these and related topics are in press. These findings are helpful in understanding factors influencing survival of pathogenic organisms within ticks and the ability of these arthropods to transmit these pathogens to man and animals.

Appendix 2. PL-480 Project 03-030-N. *In vivo* and *in vitro* Studies of Certain Tickborne Viruses in Vector and Host Cells, Al Azhar University, Cairo, Egypt.

Total Man Years: 16.25 Professional: 1.25 Other: 15.0

Annual Funding - $141,026

This project is just commencing to yield research data during its second year. It is expected to provide information concerning the sites of virus infection and replication in vector cells and tissues, and on the comparative dynamics of virus propagation in vector and vertebrate cells.



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